Silkworm Seed Technology

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Grainage Activities continued

- **Moth Emergence and Synchronization**

Moths of the component races are made to emerge on the same day, so that male and female moths are readily available for hybridization. This is referred to as synchronization.

Synchronization may be done at following levels

- **At the time of Brushing**
- **In the Market during selection - Cocoons of matching spinning dates should be purchased**
- **Refrigeration of cocoons/pupae of early batch to postpone emergence on 7^{th} or 8^{th} day of spinning at 5-10C – 7 days for males; 3 days for females**

Or

- **Refrigeration of moths of early batch at 5-10C till component strain emergence– 7 days for males; 3 days for females**

Refrigeration should be limited at only once

Effect of improper synchronization on egg hatching and quality

- **Repeated refrigeration leads to weak moths, poor fecundity, more unfertilized eggs, poor hatching, weak larvae and bad cocoon crops.**

  So refrigeration should be only once

- **It is always better to cold store male moths than female as males are more resistant to refrigeration**

Safe Period

Cocoon/Moth Stage– 7 days for male; 3 days for female at 5-10°C

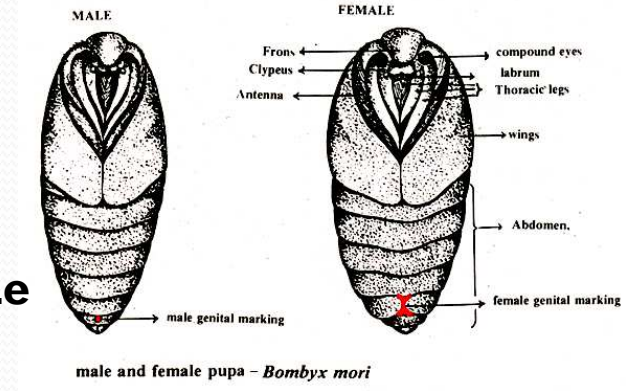
Grainage Activities continued

In the case of preparation of industrial hybrid seeds, the sexes must be separated before selfing occurs in the same parental race. This sexing may be carried out either in the larval or pupal or moth stage.

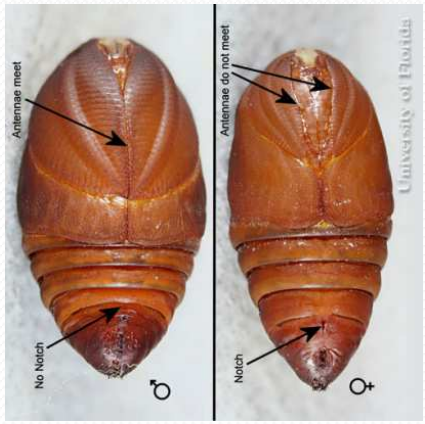
Grainage Activities continued

- **Sex Separation at Pupal stage**

Male and Female



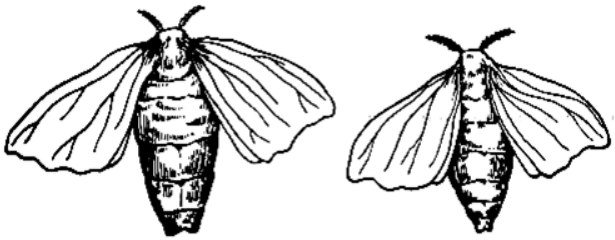
male and female pupa – *Bombyx mori*



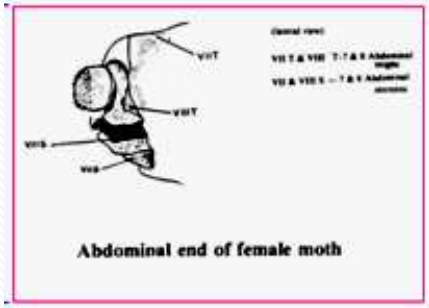
Identification of male and female pupae		
Sl. No.	**Male**	**Female**
1	Smaller in size	Larger in size
2	Narrow pointed abdomen	Broad abdomen
3	Small dot like mark on the ventral side near the top demarkation line of 9th abdominal segment	'X' mark seen on the ventral side of 8th abdominal segment

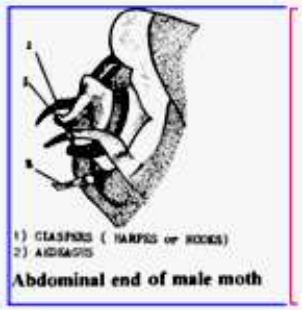
Grainage Activities continued

- **Sex separation at Moth stages**



Female and Male





Identification of Male and Female Moths		
Sl. No.	**Male**	**Female**
1	More active	Less active
2	Smaller in size	Larger in size
3	Bigger antenna	Smaller antenna
4	The caudal end has a pair of hooks known as harpes helping in copulation	The caudal end has a median knob like projection (ovipositor) with sensory hairs-which protrude d and retracted to expel pheromones

Grainage Activities continued

Coupling

As soon as the moths emerged they start to get pair.

The female moths are spread in a tray and the male moths of the desirable hybrid component are evenly distributed over the females. In about 15 minutes, the male and female moths pair.

Decoupling

After the required period of coupling (3 hours), the pairs are separated by holding the female moth and gently sliding the male. This facilitates easy separation without injury to the female reproductive organs.

Methods of Egg Production

1. Layings

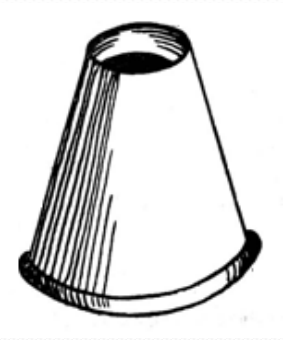
2. Loose eggs

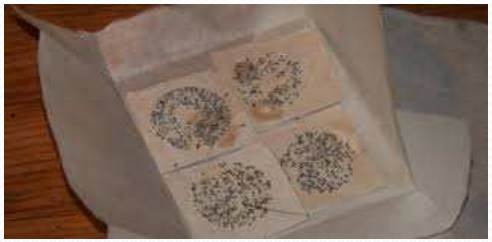
Grainage Activities continued

Oviposition:

This is nothing but the process of egg laying by the female moth.

When eggs are required to be laid on cards, the mated females are placed on the egg sheets and each moth is enclosed in a cellule. The cellule isolates the eggs laid by each moth, facilitate individual moth examination and elimination of eggs laid by diseased moth.

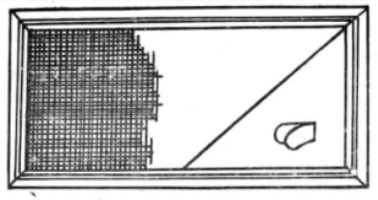




Grainage Activities continued

In case of preparation of loose eggs, a unit number of female moths are allowed to lay eggs on starched paper or cloth, with in a wooden or plastic frame. The number of moths vary from 30-200 according to the health of the batch and convenience.

Moths are allowed to lay eggs for 24 hours in a dark room with 25°C and 75-80% RH. Then mother moths are examined for the pebrine disease and eggs free from pebrine disease are qualified for rearing.



Loose Egg Container

Refrigeration of Male Moths

- **Generally it is followed for synchronization.**
- **Also, refrigeration is followed for a duration of minimum 20 minutes between the two mating during the preparation of industrial seeds only.**
- **For synchronization male moths can be refrigerated at 5-10˚for 7 days.**

Grainage Activities continued

Mother moth examination

- **Individual Mother Moth Examination**
- **Sample Testing**
- **Mass Examination of Moths**

Grainage Activities continued

Individual Mother Moth Examination

It is ideal, but laborious. Therefore is is done during the preparation of reproductive seeds.

In this case, individual moth is taken and crushed in a moth crushing set with few drops of potassium chloride solution. Then a drop of crushed fluid is taken on a glass slide with a cover glass and observed under a microscope with 6oo x magnification.

If even one moth out of 10-15 thousand moths of a batch of cocoons show pebrine the entire lot is rejected.

Grainage Activities continued

Sample Testing

In this method about 20% of the emerged moths picked at random are examined. In this case instead of crushing individual moths, two moths are crushed together. A drop of fluid is taken is taken on the glass slide, covered with coverslip and is examined under micrpscope. Even if any moth show pebrine, the entire lot is rejected.

This method is generally in vogue in India

Grainage Activities continued

- **Mass Examination**

This is quick and dependable method of moth examination for pebrine disease and practiced in commercial grainages. In this case sample moths are drawn considering the number of moths in a lot to be examined.

The methods for drawing the sample moths considering the number of moths emerged in a batch is given on the next slide

In mass examination, each sample comprises 20-30 moths.

A sample of 30 moths are ground with the help of moth crushing machine/mixer grinder with 90 ml of 0.5% potassium carbonate solution. The fluid is allowed to settle for 2 min and filtered by using an absorbent cotton filter. The fluid is then centrifuged. After centrifugation, the supernatant solution is rejected. To the sediment a few drops of 2%KOH is added and mixed well. A drop of solution is taken on a slide with a cover glass, and examined under a microscope.

At least 2 smears are examined at least 5 fields.

This method is more appropriate for large scale seed production. The norms are given in the following table

Selection of female moths for examination

Size of Batch (No. of female moths emerged)	First examination: No. of moths to be examined	First examination: No. of cups	First examination: No. of smear showkig pebrine	Second examination: No. of moths to be examined	Second examination: No. of cups	Second examination: No. of smear showing pebrine	Reject the lot if following samples show pebrine
.190 or below	390	13					1
391 to 500	390	13					1
501 to 600	450	15	1				1
601 to 700	480	16	1				1
701 to 800	570	19	2	105	4	2	2
801 to 1.000	620	21	2	130	5	2	2
1.001 to 2.000	755	26	2	195	7	2	2
2,001 to 3,000	865	29	3	500	17	3	3
3,001 to 4.000	915	31	4	815	28	4	4
4,001 to 6,000	955	32	5	1,140	38	5	5
6,001 to 10,000	990	33	6	1.500	50	6	6
10.001 to 30.000	1,030	35		1,620	54	6	6
30.001 & above	1.060	36	6	1,730	58	6	6

Dry Moth Examination.

If the eggs are to be hibernated the moths are allowed to lay eggs for 2 days and moths are tested at later days. In such cases moths are dried at about 75˚C for 4-5 hours and preserved for testing.

Drying makes the testing easier, prevents secondary contamination and kills the pebrine spores.

Environmental Conditions for Grainage Activity

Silkworm being a poikilotherm, optimum environmental conditions should be maintained for quality seed production at all stages

Cocoons are required to preserve 25°C to increase the egg production.

Beyond 32°C results in more % of melting and unfertilized eggs and also induces male sterility.

Low temperature of 20°C or below increases unfertilized eggs.

Variation leads to poor hatching and weak larvae.

Also, low humidity results in poor hatching
high RH makes the larvae susceptible to diseases
Scientific studies suggested standard temperature and humidity or gainage activities is as follows

Race	Temperature	RH
Univoltine	20-24˚C	80-85%
Bivoltine	24-26˚C	75-89%
Multivoltine	24-26˚C	75-80%

Light duration is also important as has a very important role in determination of hibernation characters. Therefore normal day light-dark conditions should be maintained.

Egg Disinfection/Surface Disinfection of Eggs

After mother moth examination, the egg sheets or loose eggs in a container are dipped in 2% formalin or 500 ppm ClO_2 with 0.5% slaked lime solution .

1. **This helps in eliminating surface contamination.**
2. **Formalin increases the adhesive capacity of eggs to the egg sheets.**

Subsequently, the sheets are washed and dried under shade and preserved under optimum temperature (25°C) and RH of 80%.

Handling of Multivoltine Eggs

- **Preservation of eggs to postpone- no need to postpone, however MV eggs can be postponed by 20 days**
- **Ideal embryonic stage for cold storage-longest embryo stage.**
- **Maximum duration of cold storage hatching maximum 20 days.**

Handling of Bivoltine eggs for early hatching

- **Multi Voltines never undergoes diapause, but uni and Bi Voltines undergo diapause or hibernation. So to check the diapause ARTIFICIAL HATCHING is done by either**

- **Physical Method - Chilling / Refrigeration**
- **Chemical Method**

Cold Acid / Hot Acid

Artificial Hatching

Hydrochlorination- Is practiced just between 15-20 hrs of oviposition. When the treatment is to be postponed the eggs are preserved at 5°C at the 20th hour. The period of cold storage should be within 5 days. During this period eggs can be taken at any time for acid treatment, but before treatment eggs are exposed to 25-26°C for 2-3 hrs.

Acid Preparation

- **HCl concentration - 40% & 1.94 sp.gr.**
- **Reduce to 1.075 sp.gr for hot acid treatment**
- **By mixing @1:1 with distilled water one before use and set to required sp.gr.**

Artificial Hatching

Hydrochlorination - hydrochloric acid treatment – Hot/Cold

1. **Hot acid treatment –HCl 1.075 sp.gr. 5-6 min at 46.1°C**

Then was the eggs in running water to remove acid traces and dry under shade.

Cold acid treatment/Room Temperature

Prepare the acid as described earlier

Sp.gr. at 15°C	2o°C	23°C	25°C	27°C	29°C	31°C	34°C	46°C	48°C
1.075	1.073	1.072	1.072	1.071	1.070	1.069	1.068	1.064	1.063
1.100	1.098	1.096	1.096	1.095	1.094	1.093	1.092	1.068	1.063
1.110	1.108	1.106	1.105	1.104	1.103	1.102	1.101	1.095	1.094

- **Room temperature acid treatment – conducted at 25°C using specific gravity 1.10 for 60-90 min.**
- **If the room temperature is slightly higher, the duration of the treatment has to be reduced according to the table.**

Age of the egg 20th – 24th hour at 24°C (Temperature during egg laying)	Dipping temperature under room temperature		
	Acid of 24°C	Acid of 27°C	Acid of 29°C
	60-90 min	60-80 min	40-50 min

Postponement of BV Hatching

- Eggs treated as above when incubated hatches in about 10 days.
- Hatching can be postponed upto 20 days as in case of MV eggs.

Acid treatment after chilling

For 50-70 days - preserve the eggs at 25C for 40-50 hrs then store at 5C. These eggs can be taken out between 35th – 50th day with intermediate temp. and treat in acid with specific gr. 1.1 at 15 °C at 47.8 C for 5-6 min.

For 40-50days - preserve the eggs at 25C for 30-35 hrs then store at 5°C. These eggs can be taken out on 30th day with intermediate temp. and treat in acid with specific gr. 1.1 at 15 °C at 47.8 °C for 5-6 min.

Hibernation

In uni & Bivoltines, the eggs enter in to diapause in 30-50 hours. Eggs when laid are yellow in colour. Gradually they change to light brown and then to purplish brown. Such eggs do not hatch until they are activated by cold temperature. This is naturally achieved in temperate countries. In tropical areas, the winter conditions could be stimulated by preserving the eggs at required temperature in a Cold Stotage.

The Schedule comproses 3 distinct phases *viz*.,

1. **Storing at 25C**
2. **Cold storage at 5 & 2.5C**
3. **An intermediate phase, 15C**

RH should be 75-80% is ideal

Hibernation schedule for 3 months

The eggs are kept at 25°C for 3 days and then cold stored for 90 days at 5°C. The cold storage and release of eggs should be through an intermediate temperature of 15°C for 6-12 hours.

Hibernation schedule for 4 and 6 months

The preservation period at different temperature is given below

Hibernation period	25°C	20°C	15°C	10°C	5°C	2.5°C	5°C	15°C
4 Months	10	2	2	3	50	50	-	1
6 Months	20	15	10	10	50	60	5	2-3

Hibernation schedule for 10 months

The preservation period at different temperature is given below

Hibernation period	25°C	20°C	15°C	10°C	5°C	2.5°C	15°C	2.5°C
10 Months	25	40	25	25	60	55	4-5	30-40

Incubation of eggs indicates the initiation of

SILKWORM REARING

Incubation

Incubation is an important process by which the activated silkworm eggs are maintained under proper environmental conditions to get hatching or preparation of eggs for hatching.

Purpose

- **To get uniform hatching with high percentage.**
- **To ensure the hatching on the required day.**
- **To ensure healthy and robust silkworms.**

Environmental conditions for incubation

For healthy development and uniform hatching eggs are to be incubated under optimum conditions of temperature, relative humidity, light and air.

Temperature

Too high a temperature, though it makes the eggs hatch earlier, results in a large proportion of eggs dying or becomes weak, hatched worms are also lighter. The cocoons spun by them are small and poor in silk content.

In short, a high temperature leads to loss of eggs, under sized worms and poor harvest.

Too a low temperature prolongs the incubation period, eggs do not hatch, and hatching is very irregular.

It is therefore, suitable temperatures for incubation of different varieties *i.e.*, Univoltines- 24-25°C, Bivoltines – 24-26°C and Multivoltines 21-24°C.

Humidity

Too high or low humidity is very bad.

Too high humidity makes the worms easily susceptible to diseases and also produces trimoulters.

Too low humidity during summer and dry seasons causes low hatchability due to embryonic death.

Therefore, it is very essential that, humidity is maintained in the range of 75-85%.

Light

Light at incubation affects the hibernating character of the eggs at succeeding generation in bivoltines. The suggested duration is 16 hours per day light is essential at incubation.

- **Air:** **The incubation area should be avoided from stagnant air. If the CO_2 content in the air increases more than 0.5% it affects development of embryo. Hence good ventilation should be maintained during incubation. The air flow in the incubation room should be 0.3m/sec, which not only brings the fresh air but also maintain the temperature and humidity to some extent.**
- **Silkworm seeds before incubation should be washed in 2% formalin for 15min, dried under shade and spread in single layer in trays.**

Paraffin Paper Method of Incubation

A sheet of paraffin paper is spread on the base of the rearing tray over which the incubation area is formed.

A second sheet of paraffin paper is placed loosely over the incubation area.

In between the two sheets, on all four sides, strips of wet foam rubber pads are placed to maintain the required humidity.

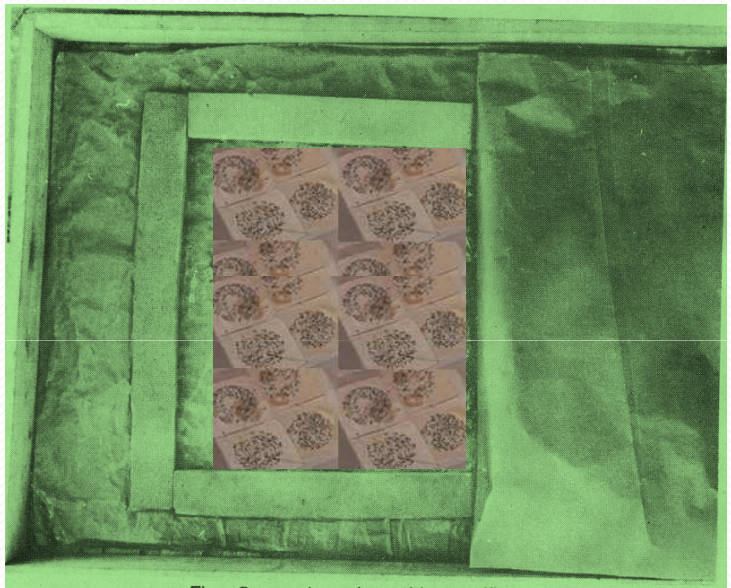


Fig. Covered rearing with paraffin paper

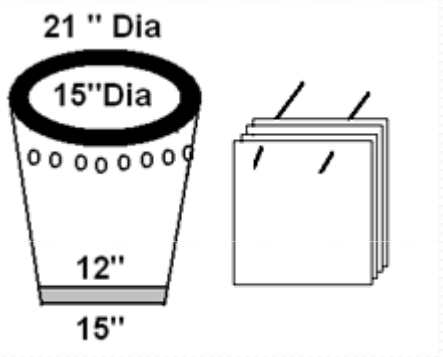
Low Cost Incubation Chamber

Mainly consists of two chambers and dimensions are shown in the picture

The walls of both the chambers are provided with 5 mm diameter holes on top half portion of the chamber, in rows in longitudinal pattern.

Cleaned and formalin disinfected sand bed of 1" depth is kept at the bottom of both the chambers. Sand in the chamber is kept moist sufficiently and conditioned at least 12 hours before keeping eggs for incubation.

The egg sheets are aligned vertically with the help of thin bamboo strips and the mouth of both the chambers are covered with loosely knit gunny cloth and the eggs are preserved till black boxing.



Black Boxing

Providing total darkness for a day or two before egg hatching is called black boxing.

This helps in uniform hatching in a single day. During black boxing those embryos in advanced stage of development will wait for light to hatch and developing embryos will continue their development and when exposed to light, all eggs will hatch uniformly.

It helps in synchronized brushing. Simple black sheet of paper (thick craft paper) or cover, which gives total darkness, is good enough.

Black Boxing:

Protocol

Eggs are pin head stage are wrapped (25 or 50 dfls each) in a tissue paper and transferred to black boxes. Such black boxes are placed under required humidity and temperature conditions. The eggs are to be exposed to light between 7 and 8 AM on the expected day (10th or 11th day) to enable maximum hatching.

Grainage Management

- **Role of LSPs/RSPs**

Silkworm seed is the backbone of sericulture industry and commercial grainages are the private bodies where disease free silkworm seeds (dfls) are produced to cater to the needs of farmers.

At present in Karnataka, the private grainages contribute to the extent of 65-69 % of the total production of dfls and the rest by the government grainages.

Bye-Products and their utilization

Like all other agro-based industries, sericulture industry also generates a lot of waste. If the waste obtained is re-cycled back, it adds to the sericulture economy. In grainage waste may be recycled back as described below

Pupa

Pupal oil extraction Feed for fish, fowls, cattles, pigs, dog biscuits *etc*.,

Cut Cocoons may be used for handicrafs

Spun silk Industry

Pierced cocoons may be used in hand spinning machine

Silk moths discarded after exclusion or mating, are now used to brew medicinal wines of ancient Chinese prescriptions

Reference/Acknowledgements to

MANUAL ON SILKWORM EGG PRODUCTION, CENTRAL SILK BOARD, INDIA 1988.
INDUSTRIAL BIVOLTINE GRAINAGE FOR TROPICS, CENTRAL SILK BOARD, INDIA 1983.