MANUAL ON BIVOLTINE REARING RACE MAINTENANCE AND MULTIPLICATION

R. K. DATTA
H. K. BASAVARAJA
YASUHISA MANO

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Supervised by :
B. S. PAPPU

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SYED MUNAWAR

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PREFACE

Sericulture, the viable agro-based industry aptly matches with the socio-economic back-drop of rural India. ***Silk, the queen of fibres*** *is the fruitful culmination of the cumulative and concerted efforts of the multi-disciplinary functionaries like cocoon producers, grainueres and reelers. Though India occupies second position in the overall global silk production (13,418 M.tonnes during 1993) bulk of it is from multi x bi silk. Its quality is at the low ebb compared to the existing International standards. Therefore, adoption of bivoltine sericulture become imperative and imminent considering its potentiality even under Indian tropical conditions. Success and development of bivoltine sericulture largely depends on practicing the specified packages, skillful management in different facets coupled with rigorous R & D support. Although, considerable information is available in this direction, practical tips/ guidelines helping to achieve the desired objectives are meagre. In line with this, considerable efforts are channelled in bringing out this manual providing relevant, precise and updated technologies of practical utility with regard to various sericultural operations for bivoltines viz., incubation, young and late age rearing, testing of silkworm hybrids, reeling test, egg production, hibernation schedules, multiplication of silkworm breeds at P4/P3/P2 levels and maintenance of silkworm stocks.*

More emphasis is given on breed maintenance and multiplication system required for bivoltines. This book also serves as necessary tool (hand book) to the technical personnel in their day to day operations. This attempt is hoped to achieve the full potentiality of bivoltine sericulture under tropical conditions and in realising the set targets thus making a significant impact in the international silk scenario besides improving the socio-economic conditions of native sericulturists and as a potential foreign exchange earner.

We express our heartfelt thanks to Dr. Y. Ohtsuki, JICA Team Leader for his guidance and suggestions. Our thanks are also due to Dr. Tanaka, Short Term Expert (JICA) in Silkworm Breeding for his valuable suggestions in bringing out this manual. We acknowledge the assistance extended by Mrs. Kshuma Giridhar, Assistant Director and Dr. M. Ramesh Babu, Senior Research Assistant. We also acknowledge the help rendered at various stages by our colleagues of Silkworm Breeding Laboratory, JICA.

R. K. Datta
H. K. Basavaraja
Yasuhisa Mano

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INTRODUCTION

The bivoltine silkworms produce cocoons with high raw silk recovery and bivoltine silk excels in quality and International standards. Originating from temperate countries these breeds have been evolved in luxuriant climate and nutrition. If these are to be raised under variable tropical conditions, one has to provide optimum conditions to realise the full competence of the breeds/hybrids. Only a small section of farmers has realised the benefits of bivoltines. Keeping in view of the optimum rearing temperature requirements for bivoltines under tropical condition, the cooler months of the year i.e., August-February are ideally suited in general for bivoltine rearings in South India when temperature is moderate with less fluctuations. The success of bivoltine crops depends primarily on the following technologies.

1.0. CRITERIA FOR SELECTION OF FARMERS

— On the basis of previous records, farmers who have harvested successful cocoon crops (bivoltine) only be selected.

— Farmers with a separate rearing house are preferred. If there is no separate rearing house, a separate room provided with windows and ventilation may be considered.

— Those who conduct systematic disinfection before and after each crop.

— Farmers having own mulberry garden with irrigation facility for raising quality leaf for young and late age worms.

— Those capable of applying adequate quantity of manure (FYM) and fertilizer.

— Having adequate rearing equipment.

— Having knowledge on the use of disinfectants and maintaining hygiene in the rearing house during rearing.

— Having wire mesh/nylon net enclosure to check uzi menace.

— Possession of a rearing house of at least 3.6m to 4.5m height preferably with false roofing.

2.0. DISINFECTION

To prevent and/or control silkworm diseases, more attention should be paid to the disinfection. The most commonly used disinfectants are formalin and bleaching powder.

— Conduct disinfection preferably at room temperature (24-25°C). The efficacy decreases with temperature less than 20°C.

— Soon after the completion of each rearing, wash thoroughly the rearing equipment, rearing house, incubation room, leaf preservation room and mounting hall with 1% bleaching powder in 0.3% slaked lime + 0.2% detergent solution or 3% formalin (Watanabe, 1995).

— Sun dry the appliances thoroughly (15-20 hours).

— Disinfect the rearing trays by dipping them into 2% bleaching powder in 0.3% slaked lime + 0.2% detergent solution or 0.3% slaked lime + 0.2% detergent

solution first and then after 30 minutes with 3% formalin by enclosing the trays, mountages etc., by a vinyl sheet.

— Avoid smearing the trays with cowdung.

— Disinfect the rearing house (Fig.1) by spray using 0.3% slaked lime solution and after 30 minutes with 3% formalin + 0.2% detergent (closed type of rearing house) or 2% bleaching powder in 0.3% slaked lime + 0.2% detergent solution at the rate of 2.5 lit/1 sq.metre floor area (open type of rearing house) (Table1).

— Provide lime wash mixed with bleaching powder (10 g lime + 1 g bleaching powder) to the walls, ceiling of the building and floor (if floor rearing is practised) soon after each rearing (Fig. 2).

— Disinfect the surroundings of the rearing house by sprinkling 10% high grade bleaching powder in slaked lime (200 g/sq.mt) for every 5-6 days.

2.1. Preparation of disinfectants

The strength of the commercially available formalin is only 36-37 % of formaldehyde. To prepare the required strength of formalin, the following formula should be used.

Method 1

$$\frac{\text{Strength of the original solution} \quad - \quad \text{Strength of formalin Solution required}}{\text{Strength of formalin solution required}}$$

This gives the parts of water to be added to 1 part of formaldehyde

For e.g. Strength required is 3 %

Strength of the original solution is 36 %

Then $\frac{36-3}{3} = \frac{33}{3} = 11$ parts

i.e., one part of formaldehyde has to be added to 11 parts of water to get 3 % formalin solution.

Method 2

$$\text{Quantity of formalin} = \frac{\text{Required concentration x Required quantity}}{\text{Available concentration}}$$

$$\text{Then to prepare 1 litre of 3 \% formalin} = 3x\frac{1000}{36} = 83.3 \text{ ml}$$

About 83 ml of commercial formaldehyde has to be added to 917 ml. of water to get 1 litre 3% formalin.

2.2. 2% bleaching powder solution

Mix 400 g bleaching powder (30% Cl_2) in 20 lts of water to obtain 2% bleaching powder solution. Filter the solution and use.

2.3. Estimation of quantity to disinfect the unit area

Before disinfection, one must find out the surface area of the rearing room to be disinfected and prepare the required quantity of formalin to cover the area. The total area of a rectangular room is calculated as hereunder

1. Floor area = Length x Breadth
2. Area of two walls = Length x Height of each wall x 2
3. Area of two other walls = Breadth x Height of each wall x 2
4. Roof / terrace area = Length x Breadth

Add up all these to get the area to be disinfected. One litre of formalin solution(2-3%) is required to disinfect the surface area of 3m x 3m (9 sq.mt). Thus, a room size of 3m x 3m x 3m requires 6 litres of formalin solution (400 ml/m^2 surface area or 2.4 litre /m^2 floor area). This is just sufficient to disinfect the empty room without any equipments. An additional quantity of 1-2 litres is required to disinfect the rearing equipment kept in such a room.

Table 1. **Requirement of bleaching powder and slaked lime for 100 dfls**

	Rearing House	Bleaching Powder (kg)	Slaked Lime (kg)
1.	Closed type with mud floor	4.340	36.993
2.	Closed type with cement/stone floor	3.270	15.123
3.	Open type with mud floor	7.966	36.630
4.	Open type with cement/stone	6.896	14.760

2.4. Fumigation

Fumigation is effective in air tight rearing house and when the temperature is higher than 23°C and under wet conditions. The fumigation in gaseous form is more effective.

In general, formalin (10%) is used for fumigation (80 ml/m^2) concentrated or diluted 4 to 5 times. Heat the formalin solution kept in a pan to liberate the formalin gas. Keep the pan containing formalin on a lighted charcoal stove filled with sufficient quantity of fuel (charcoal) to evaporate all the formalin. Close the rearing house for 24 hours. Later, open all the doors and windows of the rearing house for free circulation of fresh air before brushing.

2.5. Fumigation with paraformaldehyde

Paraformaldehyde(a polymer of formaldehyde) is a white powder, with a slight odour of formaldehyde. When heated, it activates and releases formaldehyde gas. For fumigation of 10 cubic metre, about 60 g of paraformaldehyde (also available as **Neopps**) is required.

3.0. INCUBATION

Incubation is an important step for rearing. Incubation also known as "seed warming" by which the developing silkworm eggs (embryos) are provided with proper environmental

conditions so that the embryos can develop normally and the eggs hatch uniformly. Consequently, viable cocoon crops (yield) can be attained. Wherever facilities are available eggs are to be incubated in the grainages upto the head pigmentation stage and then supplied to the farmers in a black box specially designed for it.

3.1 Preparation for incubation

Incubation may be carried out individually or collectively. An incubation centre has to be established for collective incubation. One week prior to the commencement of incubation, the chamber/room and the equipment must be thoroughly disinfected.

3.2. Transportation of eggs

The eggs are transported preferably in cool hours to prevent desiccation during hotter periods of the day. Boxes made up of wood or thermocole with adequate ventilation are preferred. Placing a wet blotting paper or sponge strip inside the box helps in increasing the humidity. CSRTI, Mysore has developed an egg carrying box (Fig.3) which is found suitable for carrying eggs for long distance (more than 100 km). To obtain good and uniform hatching of silkworm eggs, a simple egg carrying box has been designed providing optimum environmental conditions for proper embryonic development. The egg sheets are hung inside the box loosely. The bag protects the eggs from direct sunlight and shock during transportation. Water need to be sprayed over the bags at 10ml/100 cm^2. Water spraying increases humidity inside the box which helps in reducing the desiccation of eggs. In case of longer distance, it is advisable to spray water periodically.

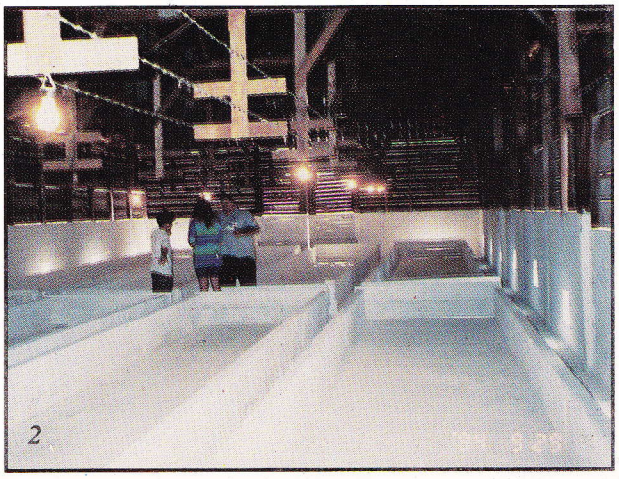
3.3. Incubation chamber/room

An incubation room is used when large number of eggs are to be incubated, wherein uniform temperature and humidity are maintained. It must also have heating and cooling devices, sufficient illumination and proper ventilation (Fig.4).

3.4. Temperature and humidity

Hibernated eggs of pure race (bivoltine) for incubation need to be released through increasing temperature gradually i.e.,10°C,15°C & 20°C for about 24 hours in each tempe- rature, whereas for hybrid eggs, an intermediate temperature of 15°C for one day is required. Then they have to be soaked in 2 % formalin for 5 minutes, washed in water and dried. The eggs are then shifted into incubation chamber and put in order by labelling i.e., race, source, batch number and date of collection.

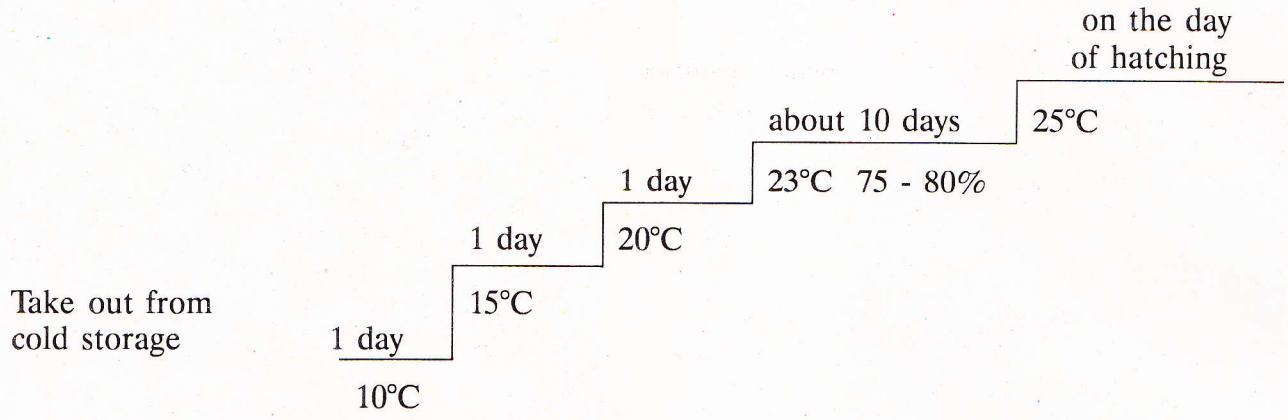
The egg cards should be spread in single layer in the trays. In the case of loose eggs, they must be spread out thinly in incubation or brushing frames. To obtain maximum hatching, optimum incubation temperature is 23°C upto 11 days and 1 day at 25°C (blue egg stage) for univoltine and 25°C upto 10 days and 1 day at 26.5°C (blue egg stage) for bivoltine (hibernated or acid treated and cold stored). The standard method of incubation of eggs is given on next page :



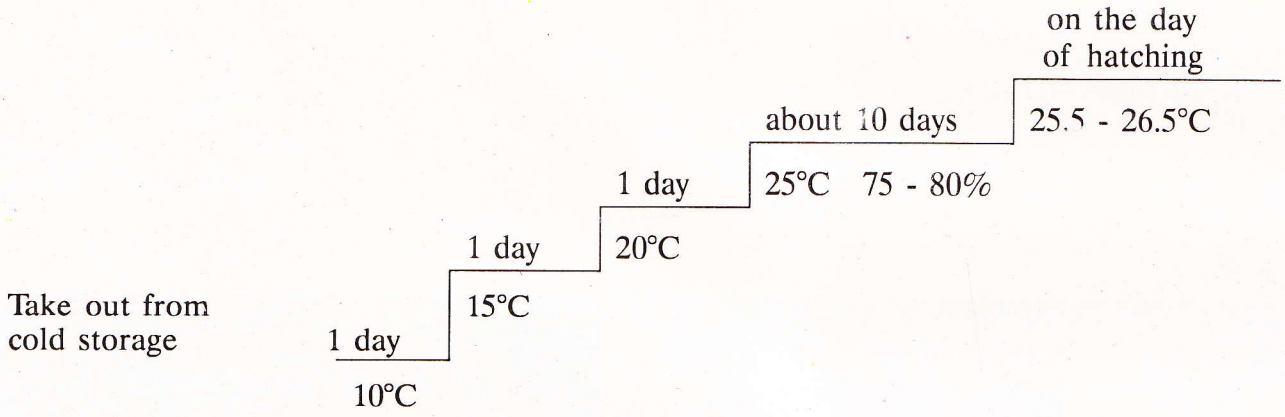
Figs. 1 to 2. Disinfection - Fig. 1 Disinfection of rearing house Fig. 2 Lime wash mixed with bleaching powder
3 to 4. Incubation - Fig. 3 Egg Carrying Box Fig. 4 Incubation room

3.5 Standard method of incubation of eggs

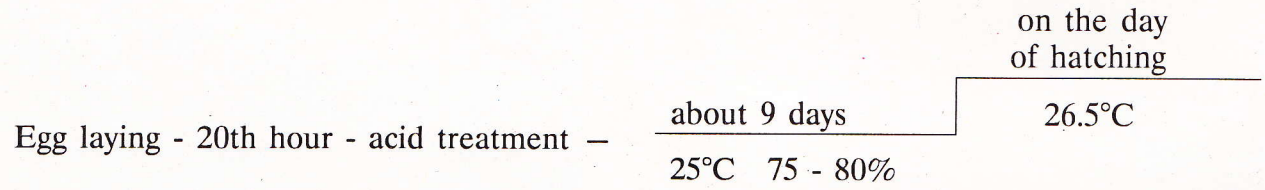
1. Hibernated eggs (Univoltine)



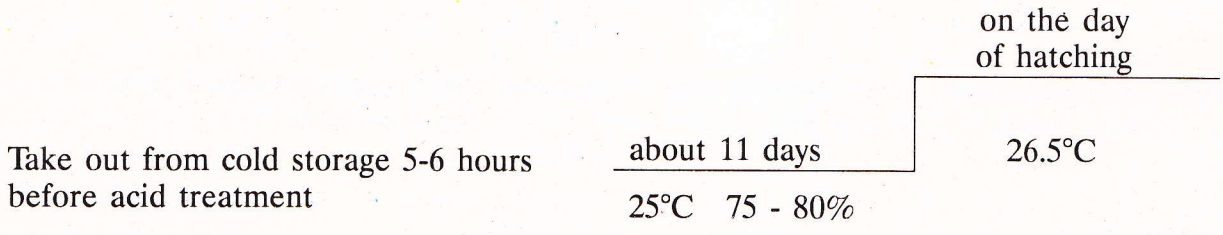
2. Hibernated eggs (Bivoltine)
   or
   Acid treated and cold stored



3. Common acid treated eggs (Bivoltine) followed by incubation (without cold storage)



4. Acid-treatment after chilling (Bivoltine)



Note: Provide 16 hours light during incubation.

During incubation, humidity is maintained at 75-80%. Excessive dryness results in dead eggs, poor hatching and weak larvae. But very high humidity (90-100 %) brings unfavourable effect on the eggs (grown larvae becomes weak).

Temperature during incubation has a great influence on the voltinism of silkworms. If the temperature is maintained at 15°C during incubation, the bivoltine breeds/races would tend to develop non-hibernating characteristics. If 23 - 25°C temperature is maintained, they tend to become hibernating ones. Rearing of hibernating eggs usually gives better crop than that of non-diapause eggs. Therefore, bivoltine eggs should be incubated at 25°C. In places having higher temperature, an airconditioner can be used for lowering the temperature while in cooler places heater with thermostatic control mechanism helps to keep a constant temperature especially in the night during incubation which is very important. As far as possible incubated layings can be supplied to the farmers.

3.6. Air

Incubation room or chamber must be opened/ventilated daily for 10 minutes in the morning and evening during incubation.

3.7. Light

The light also affects uniform hatching and voltinism during incubation period. From the very beginning of incubation upto the eye spot stage, light hastens the embryonic deve- lopment. However, during final hatching stage, darkness inhibits development. Light more than 16 hours a day induces the hibernating character in silkworms. The uniformity in hatching is obtained with regulated control of light. Light should be provided for 16 hours a day, till head pigmentation stage. At blue egg stage, the eggs should be kept in darkness by covering with black paper or cloth to obtain uniform hatching.

3.8. Exposure of silkworm eggs to light

When stray hatching is noticed on the egg card (a few larvae hatch out), the eggs are exposed to light. This ensures uniform hatching and within two hours all the larvae will hatch. The hatching date can be determined by the embryo development with reference to the colour of the egg and distinctness of the egg dimple. In case of loose eggs,the eggs are spread in the box or incubation box or brushing frame (specially meant for it) in a thin layer by shaking gently. Cutting the egg box cover cloth through the sides without damaging the eggs and keeping the same turned up with the egg sticking to it at the time of exposure to light can be done (if brushing frame is not used).

3.9. Cold storage of "blue eggs" and "new born larvae"

Hatching of eggs during incubation can be delayed by cold storing the eggs at blue egg stage at 5°C for seven days, but a shorter time (3 - 5 days) is preferable whereas, newly hatched larvae can be cold stored for 3 days at 7-10°C. The humidity in the cold storage room should not be less than 75 to 80%. Wrapping the egg or larvae container with wet cotton cloth or blotting paper helps in increasing the humidity.

3.10. Rotten eggs before head pigmentation

Sometimes rotten eggs, such as brown rot, red rot and grey rot eggs are noticed during incubation. Several factors cause this type of damage :

— Exposure to high temperature (above 28°C) and high humidity (above 90%) during egg laying and incubation.

— Storage of hibernating eggs at 25°C for too long a period (more than 90 days) under dry and high temperature conditions.

— Over stimulation of eggs during acid treatment.

— Contact of eggs by pesticides, nicotine, mosquito repellant, incense, oil or sticky material such as glue or gum.

3.11. Dead eggs after head pigmentation

Two types of embryo death can be noticed, viz., at head pigmentation stage and when eggs turning bluish (one day before hatching). In both the cases, the embryo is normally formed in the egg shell but death occurs before hatching stage. The factors for this are:

— Too high temperature during incubation (above 28°C).

— Too low relative humidity during incubation (less than 50%). This condition not only causes the death of the embryo but may also result in un-uniformity in hatching and under weight of newly hatched larvae.

— Contact of eggs with pesticides, nicotine or other toxic materials.

3.12. Brushing

Expose the eggs to light at 6:00 a.m. and brush the hatched larvae at 10:00 a.m. to avoid starvation of newly hatched larvae. In a tray of 1.2m x0.9m x0.09m size 25 dfls (bivoltine) can be brushed and reared till the end of second stage. While brushing loose eggs, spread a net of mesh size of 2 mm and give feeding. Sprinkle the chopped tender leaves of 0.5 to 1 cm size over the hatched larvae.

4.0. YOUNG AGE REARING

Usually the term young age (Chawki) refers to first two instars of silkworm rearing. Young age is the most crucial period of larval stage of silkworm as the worms are more sensitive and delicate. Hence, scientific method of young age rearing is an important component of technology of silkworm rearing which determines the success or failure of cocoon crop.

The important aspects of chawki management are suitable, separate rearing house or room (having temperature control system), well maintained garden with assured irrigation facility and adequate agro-inputs. During the first two stages, the temperature and relative humidity in the rearing room should be adjusted to maintain the range of 27-28°C and 85-90% respectively. If the temperature is too low, the physiological activities are reduced resulting in irregular growth and lengthening of the larval period.

4.1. Hygienic conditions

— Change the foot wear at the time of entering into the rearing room.

— Wash hands and legs thoroughly before entering into the rearing house each time.

— Provide separate dress for workers at their work place.

— Do not allow outsiders into the rearing house.

— Keep the rearing house and its surroundings clean and tidy throughout.

— Disinfect the feet by stepping on to the foot mat containing 10% bleaching powder in slaked lime.

— Rear silkworms on a sheet paper in rearing tray.

— Collect silkworm bed refuse into litter basket/vinyl sheet meant for the purpose.

— Dump the silkworm bed refuse in litter pit far from rearing house and mulberry garden and put bleaching powder around the pit.

— Use disinfected bed cleaning nets.

— Sprinkle a mixture of bleaching and slaked lime powder solution (1:9) in the surroundings of the rearing house periodically to prevent possible contamination.

4.2. Chawki garden

Young age silkworms require nutritious and succulent leaves. The moisture content of the leaves must be 78 - 80%. Hence, more attention should be given for increased nutrition (sugar and protein) and water content by altering the recommended dose of NPK through increasing P and K and also by adding more organic matter to the soil. Organic matter may be in the form of FYM, compost or green manure which improves the water retention capacity of the soil.

To exploit young age rearing as a commercial venture, maintain an exclusive chawki garden and follow specific pruning and harvest methods to produce maximum good quality leaves for chawki rearing alone. The important methods are:

— Prepare land in flat areas with porous fertile soil. Select sandy loam soil.

— Select S36 variety for the new garden. However, existing K2 garden with 60cm x 60cm spacing can be converted as chawki plot.

— If there is no separate chawki plot, ⅙th of the garden can be specified as chawki garden where irrigation should be repeated every 4th-5th day or sprinklers may be used every day for keeping the soil moisture status near field capacity. The mulberry earmarked for chawki should be pruned around 5 days later than the normal schedule.

4.3. Schedule of operation

After the establishment period of one year, the plants should be pruned at crown height of 20 cm above the ground level with the onset of monsoon. After 35 days of pruning, harvesting of leaves can be commenced for next 10 days (upto 2nd moult). At the end of rearing, the top terminal bud is to be clipped. After 25 days of top clipping the second leaf harvesting as shootlets (secondary shootlets) is to be done for rearing of worms upto second moult. There after, plants are again pruned at crown (80th day after first pruning). This cycle has to be repeated 4 times to get eight crops in a year (Fig. 5).

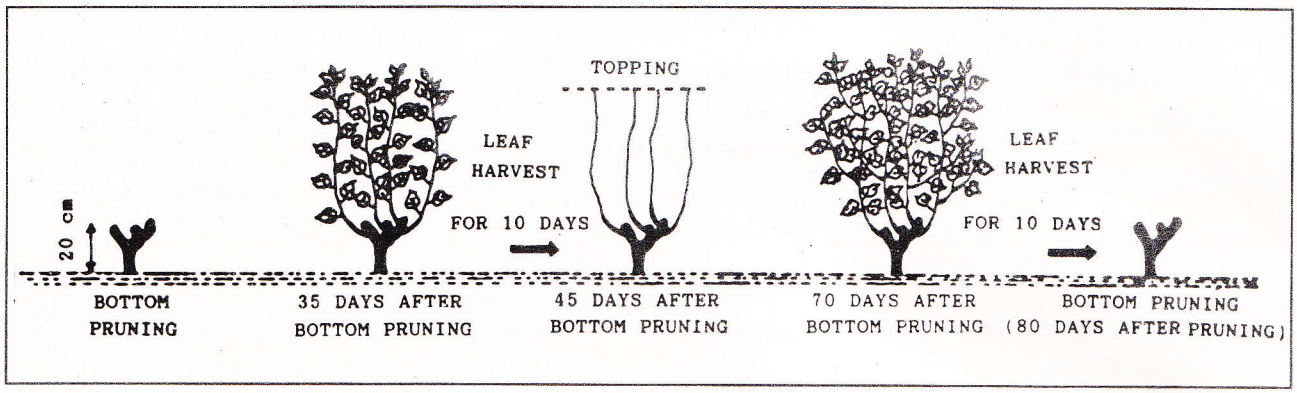


Fig. 5 Pruning schedule (cycle) of mulberry

4.4. Manure and fertilizer schedule

— Apply the annual dose of 40 M.tonnes FYM/ha in two equal splits in first and fifth crop.

— The annual fertilizer dose worked out is 225N:150P:150K kg/ha/year. This can be applied as 127 kg Suphala, 15:15:15 + 20 kg urea /crop/ha.

— Provide 3.75 hectare cm of irrigation water once in 4 to 6 days by ridges and furrows method (85 gallons of water/ha each time).

— Individual leaf plucking is recommended for first, third, fifth and seventh crops, while shootlets are to be harvested for second, fourth, sixth and eighth crops for the purpose of rearing.

— Prune the plants at the crown (20 cm above ground level) 4 times a year (after second, fourth, sixth and eighth crops) matching time schedule.

4.5. Prevention of contamination

— Keep the young age rearing room clean and tidy.

— Prevent contamination of chawki room by controlling the movement of persons and materials from outside.

— Minimise handling of larvae.

4.6. Rearing equipment

The minimum requirement of rearing equipment to rear 100 dfls of chawki worms (40,000 larvae) is as follows :

	Item	Quantity (No.)
1.	Wooden trays (120cm x 90cm x 8.9cm)	6 (Chawki)
2.	Chawki bed cleaning nets	10
3.	Litter baskets	2
4.	Paraffin paper (0.8m x 0.55m)	20 (Sheets)
5.	Hygrometer (dry & wet)	1
6.	Foam pads	16
7.	Electric room heater (with thermostatic control)	1

The important technical aspects for effective chawki management are indicated in Table 2. The desired temperature could be attained by selecting a smaller room fitted with heating arrangement and thermostatic control device. The fluctuating temperatures prevailing in day/night ultimately lead to poor growth of the fast developing worms. Consequently, humidity has to be maintained by using paraffin paper/polythene sheet whenever the room humidity is found below 80% R.H. In the absence of electrical device for heating, charcoal heating has to be done in the night whenever temperature falls below 26°C.

Table 2. **Standard chart for young age rearing of 100 Dfls (40,000 larvae)**

	Factor	I Instar	II Instar
1.	Temperature (°C)	27 - 28	27 - 28
2.	Relative Humidity (%)	85 - 90	85 - 90
3.	Leaf size (cm^2)	0.5 - 1.5	1.5 - 4.0
4.	Quantity of leaf (kg)	2.5 - 3.0	13.0 - 14.0
5.	Bed area (Sq.mtr)		
	a. In the beginning	0.36	1.35
	b. At the end	1.35	4.05
	c. No. of trays at the end (120 cm x 90 cm)	4	4
6.	Bed cleaning	-	twice

4.7. Moisture content

In newly born larvae, water content is very low, but increases rapidly upto the second instar when feeding is given.

— Silkworms require high water content in mulberry leaves to supply the needed water increment in their bodies.

— The moisture content of leaves must be above 78% and should have 30% protein and 12% or more carbohydrate.

— Variety S36 is found to be good in moisture retention capacity and suitable for chawki worms.

— Other component of increasing moisture content in leaves is regular irrigation to the plot coupled with wider spacing (0.9m x 0.9m) and higher crown height 0.2 - 0.3 m.

— For retention of moisture for longer period in leaves shoot harvest (secondary shootlets) can be practiced as being done in Japan and Brazil (commercial chawki rearers).

4.8. Quality and selection of leaves

More care need to be taken in the selection of leaves of superior quality for the young worms. The suitability of mulberry leaves for young silkworms can be determined by the position of the leaves on the shoot. Harvest one to three leaves below the largest glossy leaf (near the shoot apex) for the first instar and third to sixth leaves for second instar larvae.

Secondary shootlets (terminal bud and tender portion) are also suitable for young age rearing.

Adjustment of leaf maturity in relation to season is very important in silkworm rearing. Postponement of leaf harvest beyond 15 to 20 days during rainy season could improve the crop yield. In summer, the reverse is true.

4.9. Leaf harvest and preservation

— Harvest the leaves during cooler hours of the day preferably in the morning or evening.

— Preserve leaf for early morning feeding. Excess harvesting and long preservation should be avoided.

— Preserve leaves loosely in the leaf chamber covered with wet gunny cloth or polythene sheet in a separate storing room.

— During summer, provide fine mist of water spray on preserved leaves for better moisture retention if necessary.

4.10. Modes of rearing young larvae

The modes of rearing young larvae vary according to the equipment and climatic conditions in various regions. The common method is box rearing. This method consists of covering rearing bed of young larvae with either paraffin paper or polythene sheet. The main objective is to lower the rate of withering of mulberry leaves and keep them fresh for longer period thereby larvae get sufficient time and better micro-climatic conditions to feed .

Young age silkworms are reared at 27-28°C , 85 - 90% bed humidity with 2 or 3 feeds per day (Table 2). However, in each of these stages when the worms are under moult, the relative humidity should be brought down to 70% to dry the rearing beds.

4.11. Use of bed disinfectants

Young age worms should be reared under strict hygiene. In order to prevent grasserie and muscardine, dusting of bed disinfectant "**Reshamkeet oushadh**" (RKO) should be practised from the beginning of II instar. RKO should be dusted after every moult and covered with paraffin paper for 30 minutes before feeding. RKO should be dusted at the rate of 60 gm and 120 gm per 100 dfls in the first and second instars respectively. It is always advisable to use freshly prepared RKO preferably within 6 months .

4.12. Quantum of feeding

There is fairly good understanding to the quantitative requirement of mulberry leaf for practical rearing. The day wise feed requirement for I and II instars is given in Table 3.

Table 3. **Amount of leaf required daily in young age rearing of 100 dfls (40,000 larvae)**

Instar	Day	Leaf Requirement (kg)	Total (kg)
I	1	0.480	
	2	1.200	
	3	1.400	
	4	0.240	3.320
II	5	4.000	
	6	6.000	
	7	3.000	13.000

The standard practice is to give 3-4 feedings per day at equal intervals. Under conditioned temperature (27°C) and humidity (85%), 2 feeds per day are adequate during the first and second instars. When larvae are preparing for moult at each stage, the mulberry leaves are to be cut more finely than usual so that the unconsumed leaves dry quickly.

The appetite of the silkworm is low at the beginning of each instar, gradually increases with growth and again decreases towards the end of each stage. Therefore, the amount of feed should be regulated according to the growth of the larvae.

4.13. Cleaning

Cleaning of silkworm bed is not recommended during the first instar and cleaning is given soon after resumption from 1st moult. A net with mesh size 0.5 cm x 0.5 cm is spread over the rearing bed before feeding.

5.0. LATE AGE REARING

Ensure the availability of required quantity of rearing appliances, quality and quantity of leaf to complete the late age rearing. The minimum requirement of late age rearing equipment for 100 dfls (40,000 larvae) is given in Table 4.

Table 4. **Requirement of rearing equipment/100 dfls (40,000 larvae) for late age**

Sl. No.	Item	Quantity (No.)
1.	Round bamboo trays (1.2 m dia)	40
	or	
	Wooden trays (0.75m x 1.05m)	60
2.	Rearing stands (@10 trays/stand)	4 - 6
3.	Ant wells	20
4.	Cleaning Nets	100
5.	Feeding Stands	3
6.	Bamboo spiral mountages (1.8m x 1.2m)	40
	or	
	Plastic collapsible mountages (19 folds)	100
7.	Room Heater (Thermostatic control)	1
8.	Gator Sprayer	1

In addition to above, sufficient quantity of formalin, lime, uzicide, bleaching powder, RKO etc., should be kept in ready stock during rearing.

The 5th age larvae cannot tolerate high temperature and high relative humidity as well as poor ventilation. During this stage, larvae eat voraciously and their body water is released. Therefore, good ventilation should be given to bring down their body temperature, removal of vapours, harmful gases etc., arise from the large quantities of excreta generated due to high amount of feed consumed.

Bed spacing for the optimum larval growth has to be ensured sufficiently as indicated in Table 5 by gradually increasing the bed area everyday at the time of 9 a.m. feeding.

5.1. Temperature and humidity

— The ideal temperature and relative humidity requirement for late age rearing are indicated in Table 5.

— Manipulate the temperature and relative humidity as per the requirement using cooling, heating and humidifying appliances like air cooler, room heater, or using ash covered burning charcoal (whenever required in cooler places), wet gunny cloth, sprinkling of water to the roof, or a perforated pipe may be fitted along the crest of the roof to provide a constant water spray making wet sand beds, providing shade around the rearing house, covering the roof with thatch, using ventilators/exhaust fan etc.

— The body temperature of the silkworm is usually affected by the temperature of the rearing bed. When there is no air flow over the rearing bed, the body temperature increases with the increase in the ambient temperature or the relative humidity.

— When the ambient temperature is 33°C, the body temperature of the silkworm becomes higher than the temperature of the rearing bed by about 1°C, when the air flow is 0.1m-0.3m/sec the temperature of the silkworm body becomes cooler than that of the rearing bed. When the weather is hot, keeping the silkworm body temperature about 2°C lower than the air temperature is beneficial for the physiology of the silkworms particularly in the V instar.

— Good ventilation of air flow will help to reduce the body temperature of the silkworm. One should remember that if the airflow is too fast, mulberry leaf dries up quickly in the bed. Therefore, the airflow rate should be maintained at about 0.1m-0.3m/sec.

5.2. Feeding

The plant twigs should be 65 days old to get mature and succulent leaves. In III age, 74-75% moisture in leaf is required as against leaf with 71% moisture for 5th age worms.

— Feed the larvae sufficiently 3 to 4 times a day i.e., 5 a.m., 10 a.m., 4 p.m. and 10 p.m. Standard table for quantity of leaf and spacing for rearing of grown silkworm is given in Table 5.

— Daily feed requirement in III, IV and V instar silkworms is given in Table 6.

— The correct amount of feeding should be judged by the appetite of the worm, the amount of left over leaf and leaf drying rate in the bed.

Table 5. **Standard chart for late age rearing of 100 Dfls (40,000 larvae)**

	Factor	III Age	IV Age	V Age
1.	Temperature(°C)	25-26	24-25	23-24
2.	Relative humidity (%)	75-80	70-75	65-70
3.	Leaf size	entire	entire	entire
4.	Quantity of leaf (in kg)	45-50	175-200	1000-1240
5.	Bed cleaning	Daily	Daily	Daily
6.	Bed area in sq.mt			
	a) In the beginning	4.05	9.00	18.9-20.7
	b) At the end	9.00	18.9-20.7	37.8-43.2
	c) No. of trays (1.2m dia) at the end	10	20	40
	or			
	Wooden trays (0.75m x 1.05m)	12	26	55

— Avoid feeding of soiled, diseased, dusty and over mature leaves.

— Remove the undersized and all suspected diseased worms carefully to avoid further contamination. Collect them into 2% formalin or lime solution followed by burying or burning. With the occurrence of viral disease (NPV), change the base paper and dust R.K.O. on the new sheet and allow worms to stay there with leaf to reduce further contamination and spread of disease.

— Subject undersized worms to microscopic examination for pebrine disease.

— Do not throw the rejected or diseased larvae here and there under any circumstances. Pick out such larvae by using old news paper or unused leaf and burn them.

— Distribute the larvae uniformly in the bed during every feeding.

— Use paraffin paper (with small holes) to cover the worms during late age rearing in summer months one hour after each feeding to prevent the driage of leaf. Remove the paraffin paper again one hour earlier to next feeding.

— Feed soft leaves for about 3 -4 feedings after IV moult in summer and gradually switch over to medium leaf followed by hard leaves for two days prior to spinning.

Table 6. **Amount of leaf required daily in late age rearing of 100 dfls (40,000 larvae)**

Instar	Day	Leaf requirement (kg)
III	1	8.0
	2	15.0
	3	18.0
	4	4.0
		45.0
IV	1	10.0
	2	45.0
	3	70.0
	4	75.0
		200.0
V	1	20.0
	2	80.0
	3	110.0
	4	180.0
	5	200.0
	6	250.0
	7	260.0
	8	140.0
		1240.0

5.3. Care During Moult

— Ensure good aeration and dry condition in rearing house during moulting period.

— Spread or break the bed gently soon after the worms settled for moult to ensure drying of the bed.

— Apply slaked lime powder uniformly over the bed after breaking.

— Avoid high fluctuations of temperature and humidity as well as strong wind and bright light.

— Resume feeding when 95% of worms come out of moult.

— Use soft leaves for the first feeding after moulting.

— Apply bed disinfectant (R.K.O.) uniformly as per recommendation, 30 minutes prior to resumption from moult.

5.4. Bed cleaning

— Clean the bed once a day. Avoid hand cleaning, use bed cleaning nets.

— Examine each tray for the healthiness of worms before the commencement of cleaning.

— Remove unhealthy larvae, if any and put them into 2% formalin.

— Do not spill the bed refuse on to the floor of the rearing room while cleaning the bed. Use litter basket or gunny cloth for this purpose.

— Dispose the bed refuse in a specified compost pit which should be far away from the rearing room.

— Clean, disinfect and sun dry the bed cleaning nets after every use.

5.5. Silkworm diseases and preventive measures

Silkworm suffers mainly from four types of diseases viz.,

(1) Pebrine (2) Muscardine (3) Bacterial and Viral Flacherie
(4) Grasserie and Uzi fly pest infestation.

— Adopt strictly and effectively, the recommended disinfection practices and hygienic measures to prevent the diseases.

— Prevent contamination of rearing house and silkworm beds totally during rearing.

— Clean the floor with bleaching powder solution (5%) after day feeding during V age.

— Subject the suspected diseased larvae for microscopic examination to take suitable preventive /control measures.

— Avoid high temperature and high humidity conditions particularly during late age rearing.

— Apply bed disinfectant (Resham Keet Oushad) after every moult, before resuming the bed and 4th day of V instar after thorough bed cleaning as well as on the matured larvae before mounting.

— Make the rearing house fly proof by using nylon net or wire mesh to prevent uzi infestation.

— Use Uzi trap tablets to control uzi fly. The solution prepared by dissolving one uzi trap tablet in 1 litre of water can be effectively used to kill both the sexes of adult uzi fly. The basin containing uzi trap solution should be placed outside and inside the rearing house at the height of window-base to trap the flies.

— Destroy the uzi infested larvae and pupae immediately by burning.

— Seal the crevices in the rearing house and the appliances to prevent pupation of uzi maggots and their emergence by effective disinfection.

— Bed refuses particularly from diseased crop should not be used directly for mulberry garden. Bed refuse and other wastes should be thoroughly decomposed for a minimum of four months before use in the mulberry garden.

5.6. Shoot rearing

— This is the most economical method of rearing as labour requirements for harvest of leaf, feeding and cleaning are kept at the minimal level.

— The shoot rearing is done by providing mulberry shoots from fourth instar onwards instead of individual leaves. The shoot rearing is practiced in sericulturally advanced countries like Japan, China, Russia and Brazil throughout the year.

— Irrigated garden is suitable for this method. Under normal condition, 40-50 days growth period i.e., from completion of one crop till the beginning of young age (second crop) is required.

— A separate rearing house nearer to the garden may be constructed for this purpose.

— The rearing house should be made rodent and uzi proof with an anti-chamber, shoot storing room and chawki room.

— In shoot rearing system, soon after third moult the larvae are shifted to rearing shelves for shoot feeding (Fig. 6 & 6a).

Fig. 6 & 6a Shoot rearing

- The ideal size of each shelf of the rack shall be 1.5m width, 10.5m length. The shelf should be provided with 15 cm border to prevent the falling of larvae. In each shelf 20,000 larvae or 50 dfls can be reared upto spinning.
- These shelves can be arranged in two tiers with a gap of 0.75m or 3 tiers with an interval of 0.70m distance.
- About 65 days old shoots are cut 5cm-7.5cm above the previous cut. The height of the shoot at the time of harvest will be 0.9m-1.2m.
- The harvested shoots are transported in the form of bundles of 25-30 kg. The shoots are stored vertically upright on a clean floor and covered with wet gunny cloth.
- The shoots are spread everyday on the bed width-wise (perpendicular to the long axis). The shoots are placed in such a way that the top and bottom ends of the shoot are placed alternatively to ensure equal mixing of different quality of leaves in the bed.
- The final spacing required for 100 dfls under shoot rearing method is 27.0-31.5 sq.m as against 43.2 sq.m in leaf rearing method.
- Bed cleaning is done only once i.e., on the second day of the fifth instar by using rope or net.
- In case of rope cleaning method, two ropes of 1.8m length are spread parallel to one another for a length of 1.5m on the bed, leaving a margin of 0.45m on either side. After three feedings when larvae come on to the top layer of the shoot, the ends of the rope are pulled to the centre so as to make a bundle. This helps in separating the old bed easily without causing damage to the larvae.
- In net cleaning method, about 5cm x 5cm mesh size nets of 1.5m x 1.8m have to be spread over across the bed. After 2-3 feedings over the net they are lifted and the old bed is cleaned.

6.0. MOUNTING AND HARVESTING

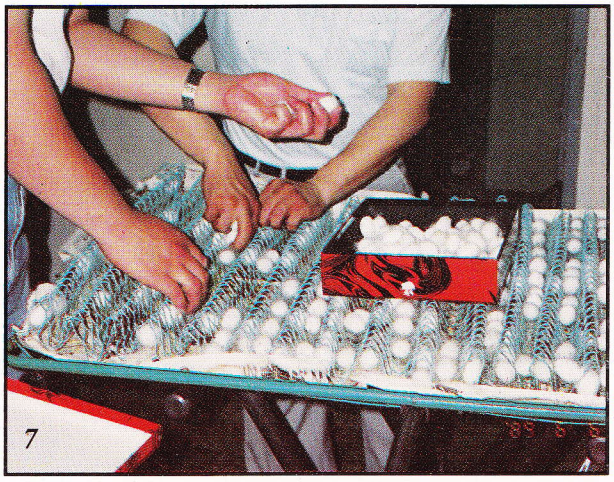
Silkworm reaches the peak growth in six to eight days after they enter the fifth stage and within a day they grow into matured silkworm ready for spinning the cocoon. At this point they are considered mature and are put on the mountages for spinning the cocoons. This process is called mounting.

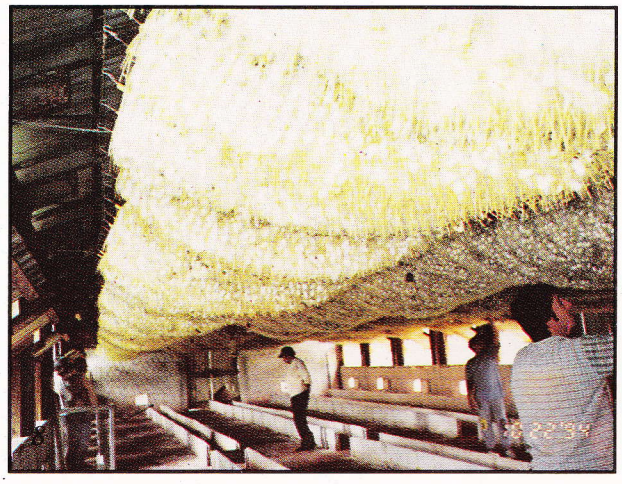
The mature silkworm is readily distinguishable by its translucent colour. The body colour becomes yellow and also shrinks in length. Faeces become greenish, soft and irregular in shape. The silkworms stop eating and raise their head and thorax. Generally, the ripened worms move towards the periphery of the rearing trays in search of anchorage to spin the cocoons.

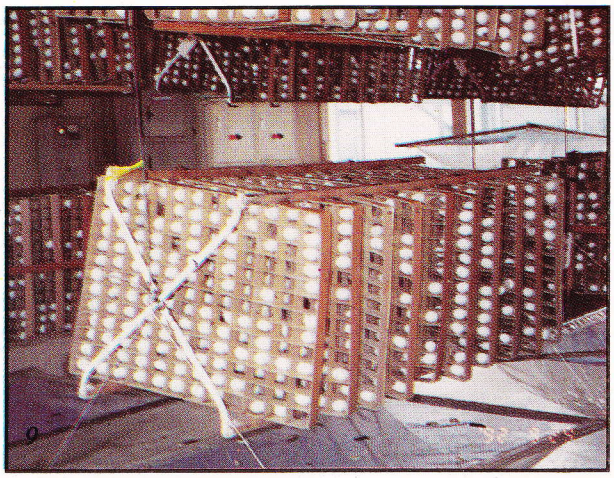
6.1. Mounting of spinning larvae

- The mounting and cocoon harvest operations are the final steps in silkworm rearing. If these operations are not handled properly, the quality of the silk may be adversely affected and 25 days hard work carried out earlier will be a waste.
- Reduce the feeding quantity and leaf size when spinning commences, but the frequency of feeding may be increased in the penultimate day.

- Arrange the required number of mountages well in advance and also mount the worms when they are fully mature. Any negligence at this stage is bound to cost the rearer heavily.
- Mount spinning worms by hand picking or shoot shaking followed by self mounting. Each method has its own advantages and disadvantages.
- Pick the matured silkworms individually by hand in hand picking method. The advantage of this method is properly matured worms alone can be collected. This method is time consuming.
- In the case of shoot shaking method, when 30% of the silkworms have become matured, the shoot to which they are attached is shaken by hand or vibrated mechanically to shake off the silkworms and to collect them on a cocooning frame.
- During self mounting, silkworm larvae attached to mulberry shoots are shaken before optimum mounting stage and the larvae are again reared on a bed. Then at the time of mounting, the cocoon frame is placed over the rearing bed so that the matured larvae can climb up. This method is advantageous because the labour requirement is not more on the day of mounting. However, the disadvantages are that after shoot shaking, mulberry harvest or feeding, space and labour are needed for preparing new rearing beds.
- Use only clean and disinfected mountages.
- Mount about 30 - 40 larvae /sq.ft.
- Use bamboo or plastic bottle brush or plastic collapsible mountages for seed cocoon/ reeling cocoon production. However, in order to decrease the defective cocoons, such as soiled cocoons, mountage pressed cocoons, double cocoons etc., to improve the cocoon quality and to facilitate the adoption of free mounting method for the purpose of saving labour and to increase the reelability of commercial cocoons use rotary card board mountage (Figs. 7-9).
- Cover the plastic mountages with nylon or cotton net soon after mounting (Basic stock multiplication centre and Silkworm Breeding Lab) to avoid mixing of larvae (Fig. 10).
- Place old newspapers under the mounting frame to absorb urination and excessive moisture in the mounting room.
- When 80% of the larvae have been completed web formation (hybrid 20-24 hr. and parent race 24-48 hr), without shaking the plastic mountage, gently remove the base paper and place the mountage upside down. This improves the reelability of the cocoons.
- Remove diseased and dead worms while taking out the paper placed on the base.
- Record shrunken larvae (non-spinning) noticed on the mountages. (If non-spinning larvae are more, transfer them to new mountages).
- Mounting of premature and over mature larvae affects the quantitative traits of the cocoon. The effect of early and late mounting on cocoon characters is given in Table 7.







Figs. 7 - 10 Different Types of Mountages

Fig. 7 Plastic collapsible mountage
Fig. 8 Plastic bottle brush type mountage
Fig. 9 Rotary mountage made of cardboard
Fig. 10 Plastic collapsible mountages covered with nylon net

INFLUENCE OF TEMPERATURE ON PUPATION AND EMERGENCE

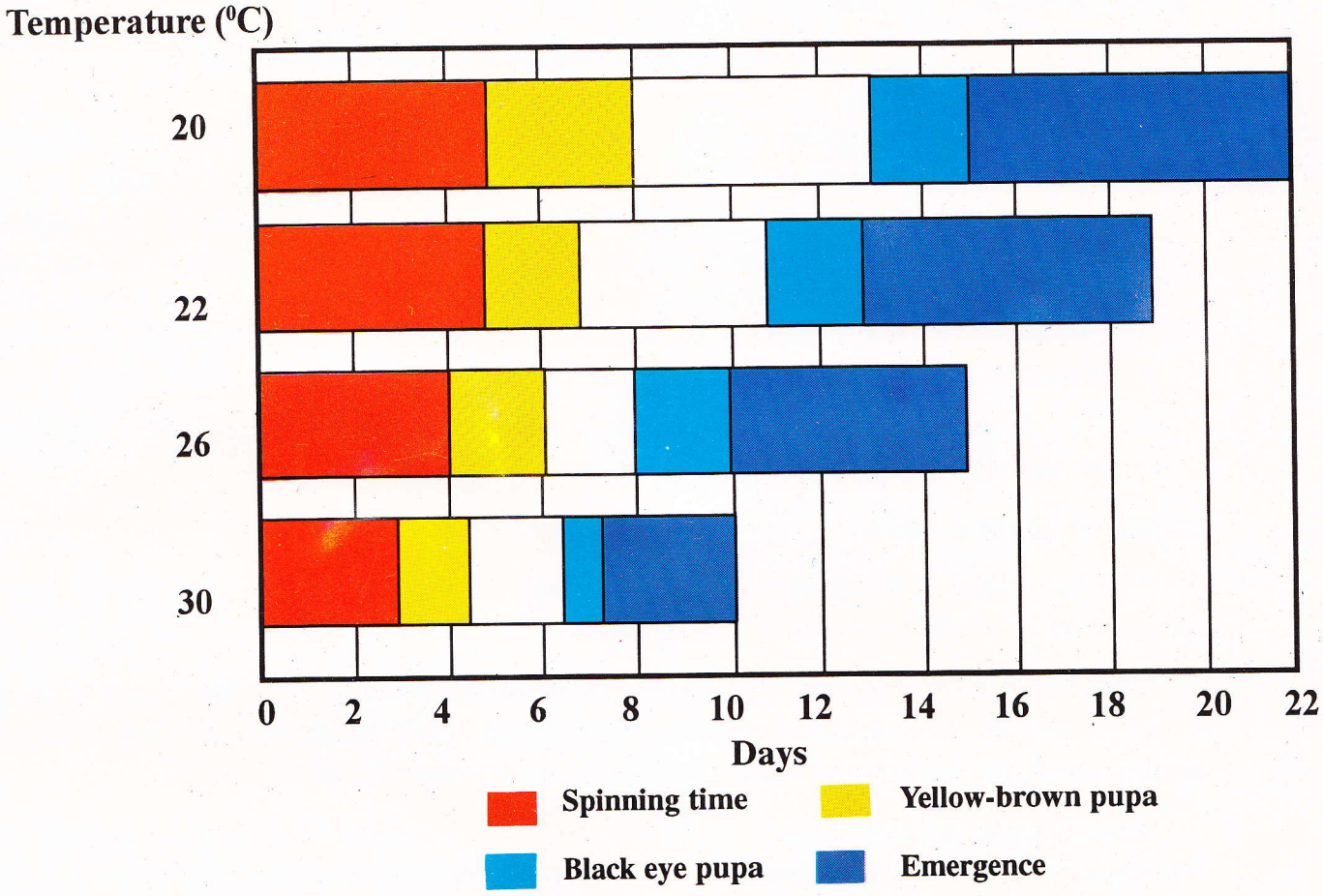


Table 7. **Effect of mounting time on cocoon characters**

Mounting time	Cocoon weight (g)	Shell weight (cg)	Raw silk (%)	Filament length (m)
Just mounting time	1.70 (100)	38.5 (100)	18.9	1,112
Before 12 hours	1.71 (101)	38.9 (101)	18.7	1,159
Before 24 hours	1.64 (96)	36.8 (95)	17.7	1,063
Before 36 hours	1.53 (90)	31.5 (82)	17.1	970
Before 48 hours	1.46 (86)	30.4 (79)	16.8	969

Gumma sanshi: T. Saito, 1992

- Maintain the temperature of 25 to 26°C and the humidity at 60 to 70% during mounting. Care must be taken to avoid the temperature rise beyond 28°C and humidity increase to more than 80%. Such high temperature or humidity is detrimental to the quality of cocoons. Since temperature and humidity during the initial 92 hours after mounting exert greatest influence on the cocoon quality, attention must be given to control the conditions. If the temperature is less than 20°C and humidity is high, use electric heater to raise desired temperature.
- Provide continuous air current during spinning and that helps to remove the excess moisture and improve the reeling qualities of cocoon.

6.2. Harvesting of cocoons

The harvesting of silkworm cocoons is the final stage of the process of silkworm rearing and must be carefully carried out.

The best time for harvesting of cocoons must be decided correctly . If it is too early, the larvae might not have pupated, the skin of the pupa is still tender and easily be damaged, staining inside the cocoon shell and affecting the quality and colour of the silk.

Harvesting of cocoons during cooler months (winter) is performed in **seven to eight days** after mounting, whereas in summer or autumn rearing it is conducted in **six to seven days** after mounting. It is advisable to cut open some of the cocoons and check the pupation before harvesting. Assessment of cocoons to be carried out on the next day. The optimum time for harvesting is when the pupa turns brownish in colour and its integument becomes hard. Remove dead worms, flimsy and stained cocoons from the mountages before harvesting good cocoons.

Morphological changes of pupa at different temperature and days required for emergence is given in Fig.11.

6.3. Cocoon assessment

Next day after harvesting, deflossing of cocoons is to be carried out (Fig.15). Then sorting of cocoons (i.e., melted, thin shelled, uzi infested, deformed and double cocoons) is to be done (Fig.12). These defective cocoons are to be recorded systematically in the data sheet. After sorting, by gently shaking each cocoon for live pupal sound, cocoon number (pupation rate) is to be calculated. The number of dead pupae cocoons is also to be recorded. Count the live cocoons

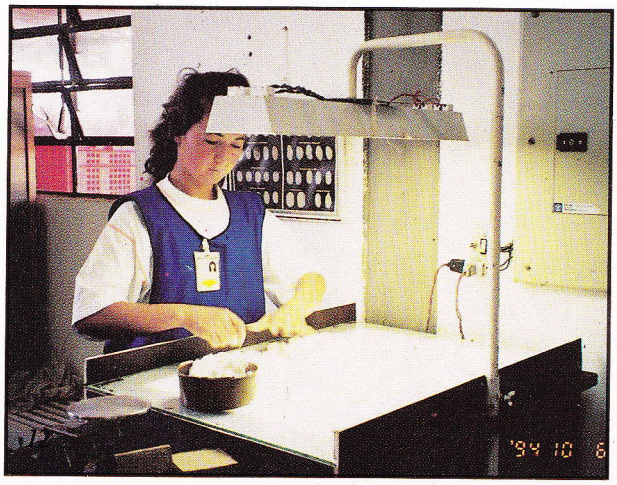
by using cocoon counter specially meant for it. Then actual weight of counted cocoons should be recorded.

Divide the batches (each bed) into two equal halves. From one half, take 50 cocoons from cellular batch and 50 to 100 cocoons from hybrid batch at random. The use of cocoon counter is recommended. The cocoon assessment is done by using electronic balance (Fig.13).Cut the cocoon shell and sex the pupae for calculating the average cocoon weight, cocoon shell weight and cocoon shell ratio of both male and female separately (Fig.13a &13b).

After the completion of total procedure, from the remaining half, 50 reelable cocoons in the case of cellular batches and 300-1000 cocoons in case of hybrids should be selected at random and weighed. These cocoons should be sent for reeling test.

6.4. Points for attention

- Spinning may last for 2-3 days. During this period the reelability of cocoons and the quality and quantity of silk produced are closely linked to the environmental conditions.
- Temperature influences the speed of the spinning worms and quality of silk. Higher the temperature, more the rapid excretion of silk bave and vice-versa.
- If the temperature rises too high, the cocoon-shell will become very loose and folded with wrinkles and knots. It will increase the cohesive power of silk filaments, causing more difficulty in reeling and increasing the number of dropped ends.
- The mature larvae will excrete more urine during spinning and create a humid environment in the mountage. If the humidity is too high, more larvae and pupae will die. It is difficult for the cocoon-shell to become dry. Since, the filaments of fibroin adhere tightly to one another, it is hard to dissolve the sericin, making reeling more difficult.
- If the atmosphere is too dry, the cocoons will be defective (multi-layered cocoons, loose end cocoon shell etc.), the filament will break easily, difficult for reeling and lower the neatness of the silk.
- The optimum relative humidity for mounting is 60 - 70%. In high humid areas, it is extremely important to expel excessive moisture during the mounting period.
- In practice, it has been proved that high temperature (27-28°C) and good *air current* can increase the reelability significantly.
- If premature larvae are mounted, they will crawl without cocooning for long time. This not only is trouble some for mounting management, but also cause low raw silk percentage of cocoons later on.
- Mature silkworm larvae have a tendency to turn away from light. When strong light is provided in the mounting room, the larvae crawl to one side, forming many double cocoons or cocoons of uneven thickness. Provide light (10-20 lux) in the mounting hall.
- The double cocoons are mainly due to inadequate space for spinning, over crowding of worms on the mountage and mounting of prematured larvae.
- Double cocoon formation is also a characteristic of certain silkworm breeds.



Figs. 12 - 13b Assessment of Cocoons.

Fig.12 Cocoon sorting Fig.13 Single cocoon assessment by electronic balance with computer print out
Fig.13a General cocoon assessment (mass) Fig.13b Single cocoon assessment

6.5 Parameters for investigation

Proforma for collection of rearing data,cocoon assessment and reeling test is given below.

Parent race rearing (for each cellular batch)

a) Race name : XX

b) Log No. :

A. Present : 110

B. Source (95 x 98) : 100

c) Date of brushing : 25/11/92

d) No. of DFLS : 1

e) Eggs/laying : 600

f) Larvae brushed : 550

g) Hatching % : 91.66 = 91.7

$$\frac{\text{Hatched egg No.}}{\text{Total egg No.}} \times 100$$ (correct the hatching % to one decimal)

Note : Total egg No. = Hatched egg No. + unhatched egg No. + unfertilized egg No.

h) Larvae after III moult (Basic No. of larvae) : 300

i) Larval period : D : Hr

a) V th stage : 6 : 23

b) Total : 24 : 06

j) Larval weight (g) : 55.6 (random sample of 10 larvae) (V th age peak growth)

k) Actual Yield :

(i) By No. : 281 (good cocoon + double cocoon)

(ii) By wt.(kg) : 0.426(good cocoon + double cocoon)

l) Yield/10000 larvae :

(i) By No. :

$$\frac{\text{9461 (Actual yld. by No.)}}{\text{(Basic No. of larvae-Uzi infestation)}} \times 10{,}000$$

(ii) By wt. (kg) : 15.16 = 15.2

$$\frac{\text{Actual yld. by wt.}}{\text{(Basic No.of larvae-Uzi infestation)}} \times 10{,}000$$

m) Cocoon/Litre (No.) : 75

n) Single cocoon wt. (g) : 1.516 = 1.52

(Mass cocoon assessment) (Average weight of 25 females and 25 males)

o) Single shell wt. (g) : 0.423

(Mass cocoon assessment) (Average Weight of 25 females & 25 males)

p) Shell ratio (%) : 27.90 = 27.9 $\frac{\text{(shell wt.)}}{\text{(cocoon wt.)}} \times 100$

q) Melting % : 3.0 $\frac{\text{(Missing larva No. + Melted Cocoon)}}{\text{(Basic no. of larvae - Uzi infestation)}} \times 100$

r) Double cocoon % : 0.2 $\frac{\text{(Double cocoon number X 2)}}{\text{Total cocoon number}} \times 100$

Reeling parameters

(The data calculation must be carried at reeling sections/units only)

Note: Reelable cocoon weight/reelable cocoon number = Single reelable cocoon wt.

1) Filament length (m)	:	1100
2) Filament wt. (g)	:	0.359
3) Filament size (d)	:	2.937 = 2.94
4) Reelability (%)	:	85
5) Neatness (point)	:	95.0
6) Raw silk (%)	:	23.6
7) Boil-off-loss (%)	:	24.6
8) Other characters (if any)	:	

Qualitative Parameters

a) Larval body colour	:	Blue/Red/Reddish/Yellow/Light Brown
b) Body shape	:	Slender/Medium/Stout
c) Larval marking	:	Marked/Plain/Faint marking/Sex-Limited
d) Larval blood colour	:	White/Yellow
e) Cocoon colour	:	Pure White/White/Cream/Rusty/ Green/Golden Yellow
f) Cocoon shape	:	Dumbbell/Peanut/Constricted/Elliptical/Oval
g) Cocoon uniformity	:	Excellent/Good/Medium/Slight variation/ Not uniform
h) Cocoon grain	:	Fine/Medium/Coarse/Fluffy
i) Cocoon trunk	:	Hard/Normal/Soft
j) Cocoon end	:	Hard/Normal/Soft

7.0. TESTING OF SILKWORM HYBRIDS

- The hybrids which were identified by the breeders in the laboratory have to be subjected for systematic testing at different centers.
- Research institutes and Universities involved in silkworm breeding should conduct the testing of newly evolved hybrids systematically in the proper environmental conditions. Researchers are responsible persons in their research work and should obtain good results which ultimately help the sericulture industry. Hence, it is necessary that the researcher should understand his responsibility towards the usage of his research results.
- The committee constituted for authorising the silkworm breeds/hybrids should review the experimental results thoroughly and the committee also should take decision whether the breeds/hybrids can be authorised or not. Further, all the results of the experimental research should be collected and kept in Research Institutes for reference.

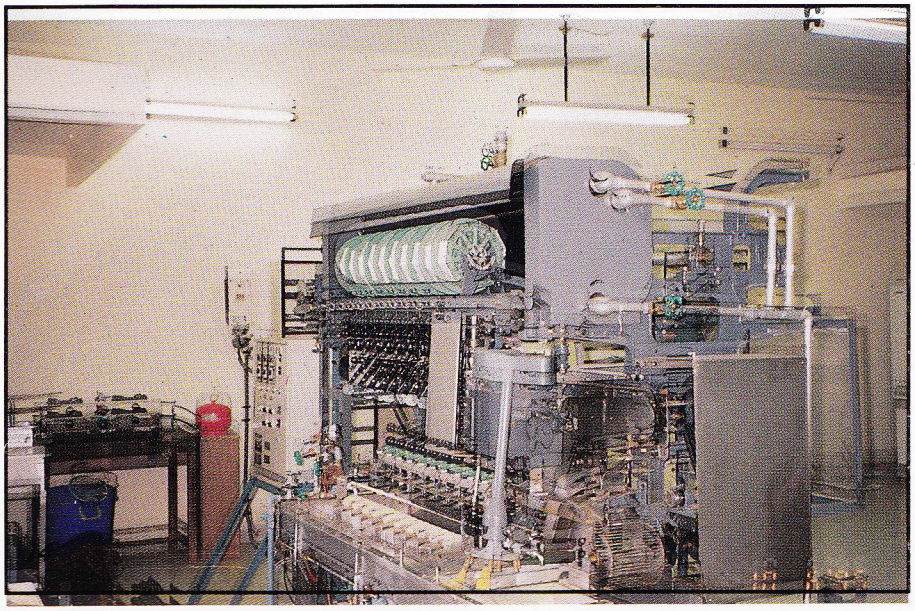


Fig.14 Multiend reeling machine Fig.15 Deflossing of cocoons

- Testing of breeds/hybrids under stress conditions (high temperature, disease tolerance and poor quality of leaf) should be enforced by special breeds having tolerance.
- RSRS's, REC's and TSC's should conduct adaptability test with regard to authorised hybrids and identify suitable hybrids for respective climatic conditions and they should give guidelines while popularising the selected hybrids at farmers level.

7.1. Investigation method

- Before authorisation, the identified hybrids are to be subjected for testing at RSRS/REC/ BSF of main Institute. In each centre 10 dfls are to be reared.
- 10 Dfls of control hybrids should also be reared along with new combinations.
- Approximately 4000 silkworm larvae should be brushed (each hybrid) and after III moult 2500 larvae should be retained.
- After harvest, calculate the survival rate.
- 100 female and 100 male cocoons taken at random for calculation of average cocoon weight, cocoon shell weight and cocoon shell ratio.
- From the remaining cocoons select reelable cocoons and subject them for test reeling results (CSTRI, Bangalore or other test reeling centres) by reeling with automatic or multiend reeling machine (Fig.14).
- The rearing and reeling data should be collected and compiled by the main Institute.
- Subject the consolidated data to silkworm race authorisation committee for discussion.
- Out of the testing places selected half of the centres should test straight crosses (A X B) and other half of the testing centres should test reciprocal hybrids (B X A).

7.2. Proforma for collection of hybrid data

Rearing test

1. Experiment/test No.
2. Name of the breed/hybrid
3. Mother strain (Dumbbell or oval)
4. Egg (hibernated, non-hibernated, immediately acid treated and acid treated after chilling)
5. Incubation : No. of days of cold stored, cold stored at blue egg or hatched larvae.
6. Date of brushing :
7. Rearing method : Shelf/shoot

8. Rearing environment : I, II, III, IV, V instar Temperature (°C) and Humidity(%) Environmental condition good/bad /fair Season : Rainy (days) /Winter (days)/ Summer (days) Leaf quality (Season wise)

9. Mounting method : Self/picking

10. Mountage used : Bamboo spiral, plastic collapsible or plastic or bamboo bottle brush.

11. Mounting condition : Temp (°C), Humidity (%) during and after mounting (4 days).

12. Hatchability % : 94.23 = 94.2

$$\frac{\text{Hatched egg No.}}{\text{Total egg No.}} \times 100$$ (correct the hatching % to one decimal)

13. Basic larval No. : (Larvae after III moult)

14. Larval duration (I, II, III, IV, V instar and Total) (D : H)

15. Missing % : IV & V stage, on the mountage and Cocoon Stage

16. Pupation percentage of 3rd ecdysed larvae (uzi infested silkworm should be deducted from the basic larval No.) : $\frac{\text{Number of pupae/}}{\text{standard number of silkworms}} \times 100 = 95.1\%$

17. Trimoulter larval No. :

18. Non-spinning larval No. :

19. Others (if any) : Opinion about the general situation during rearing and spinning time.

20. Cocoon yield (kg) : Single cocoon weight (reelable cocoon) X 10,000 = (19.8 kg)

21. Cocooning % :

a) Good cocoon = $\frac{\text{Good cocoon (reelable cocoon)}}{\text{Total number of cocoons}} \times 100 = 98.52 = 98.5$

b) Double cocoon = $\frac{\text{Number of double cocoons}}{\text{Total number of cocoons}} \times 2 \times 100 = \frac{11 \times 2}{1852} \times 100 = 1.19$ or $= 1.2$

22. Cocoon/litre :

23. Cocoon weight (g) (2.15 g)

24. Cocoon shell weight (g) (0.45 g)

25. Cocoon shell ratio (%) (21.0)

Note: For assessment take randomly 100 females and 100 males, then calculate the average.

26. Qualitative parameters

a) Larval body colour	:	Blue/Red/Reddish/Yellow/Light Brown
b) Body shape	:	Slender/Medium/Stout
c) Larval marking	:	Marked/Plain/Faint marking/Sex-Limited
d) Larval blood colour	:	White/Yellow
e) Cocoon colour	:	Pure white/White/Cream/Rusty/Green/ Golden Yellow
f) Cocoon shape	:	Dumbbell/Peanut/Light constricted/Elliptical/ Oval/Spindle/Squared type
g) Cocoon uniformity	:	Excellent/Good/Medium/Slight Variation Not uniform
h) Cocoon grain	:	Fine/Medium/Coarse/Fluffy
i) Cocoon trunk	:	Hard/Normal/Soft
j) Cocoon end	:	Hard/Normal/Soft

27. Quantity of cocoons for test reeling

1,000 reelable cocoons along with data to be sent for test reeling centre

a) Log No. (Test No.)	:	
b) Race name	:	
c) Date of spinning	:	
d) Spinning environment	:	Temp. (°C) and Humidity (%)
e) Reelable cocoon number	:	
f) Reelable cocoon weight	:	(i) Total green cocoon wt.(kg) (ii) Single green cocoon wt.(g) (iii) Single dry cocoon wt. (g) (iv) Drying (%)
g) Single cocoon wt.(g)	:	
h) Single shell wt.(g)	:	
i) Shell ratio (%)	:	
j) Date of stifling	:	
k) Stifling environment	:	(i) Hour (Hours : Minutes) (ii) Maximum temp. (°C) (iii) Minimum temp. (°C) (iv) Average temp.(°C)

8.0. COCOON DRYING

The primary objectives of drying cocoons are to kill pupae before they emerge and to kill maggots of parasitic moths and flies if any, before they emerge from the pupae.Secondary objectives are to reduce the water content of fresh cocoons to minimise the chances of fungal infection during the period of storage so that the quality of cocoons becomes suitable for subsequent processing.

8.1. Hot air drying

The best method of cocoon drying is done at different temperatures for 6 hours. The weight of green cocoons is recorded before drying.

Drying of cocoons is generally done by setting the initial temperature with the range of 95-60°C and then by lowering the temperature gradually as drying progresses. Finally, drying is completed at the temperature of around 60°C.

The procedure adopted for green cocoon drying is detailed below.

1) At the first one hour — 95°C (drying temperature)
2) At the second hour — 93°C
3) At the third hour — 90°C
4) At the fourth hour — 85°C
5) At the fifth hour — 75°C
6) At the sixth hour — 60°C

In step wise drying, one should check the temperature every one hour following the above mentioned standard method. At the beginning the moisture in the cocoon is very high, so that high temperature is preferred. After one hour of drying at 95°C, moisture in the cocoon is decreased, then low temperature is suitable for cocoon drying.

After five hours of drying, the first cocoon weight should be recorded. The second cocoon weight should be recorded after drying again at 60°C for 30 minutes. If the first and second cocoon weight found same, one can assume that drying is completed. Then the percentage of the driage may be calculated. However, the standard drying percentage is calculated from cocoon shell ratio.

9.0. REELING TEST

The test reeling centres have to process the cocoons as mentioned below.

Out of 1000 reeling cocoons, first 600 cocoons to be reeled are taken at random and divided into two batches of 300 cocoons each. All the reeling parameters are to be studied. In case, for any character, if the deviation of the results in the two batches is significant, then the third batch of 300 cocoons are to be reeled again and the results are obtained. Then average is calculated. The remaining 100 cocoons are to be used only for cooking test.

Note : *300 : 300 : 300 : the cocoon weight should be taken and the weight to be made equal by interchanging the cocoons among them.*
The results obtained out of three batches, the batches which are showing characters nearer only to be taken for calculation of average data.

9.1. Reeling assessment method

	MULTI-END.	AUTOMATIC
1. No. of reeling ends	4	3
2. Reeling speed	80m/min.	80m/min.
3. Carry over cocoon (No.)	32	60
4. Carry over end	1	1
5. Reeling water temp.	38°C	40°C
6. Reeling cocoon number	8	-
7. Size of denier indicator	22 - 24 (d)	27 (d)

9.2. Reeling traits

1. Length of cocoon filament (m) (1250 m)
2. Weight of cocoon filament (g) (0.385g)
3. Size of cocoon filament(d) (2.06 d)
4. Raw silk percentage (%) (17.9%)
5. Reelability (%) (84.0%)
6. Neatness points (40 panels) (90p)
7. Neatness low grade points 1/5 low grade panels average points. (85p)
8. While recording silk weight, tenacity, elongation and length, the room temperature and humidity should be 20°C & 65% respectively.
   a) Exfoliation test - grading points (80p)
   b) Tenacity (g/d) (40)
   c) Elongation (%) (22%)
   d) Cohesion(s) (69s)
   e) Eliminated cocoon (%) (2.1%)
   f) Degumming loss(%) with reference to cocoon shell 24.0%
      and for raw silk 18.5%.
9. The average data for the traits viz. non-breakable filament length, renditta and raw silk percentage of cocoon shell should be recorded.
   a) Non-breakable filament length of a cocoon filament length X reelability (m) (1050m)
   b) Renditta = 1/raw silk percentage(kg) (5.6kg)
   c) Raw silk percentage of cocoon shell weight of cocoon filament/cocoon shell weight X 100 (85.4%)

The formula for calculation of reeling character is given below :

9.3. Example for calculation of reeling characters

1. Reelability percentage = $\frac{\text{number of reeling cocoons}}{\text{number of feeding end}} \times 100$

   a. Number of reeling cocoons : testing cocoons - number of unreeled cocoons - number of carry over conversion cocoons

   b. Number of feeding end : measurement of feeding end + number of carry over cocoons - number of carry over conversion cocoons.

e.g. Testing cocoons = 300 ; measurement feeding end = 337
Unreeled cocoons = (fully 1, middle 3)
Carry over cocoons = (outside 26, middle 27, innerside 7) (auto)
Carry over cocoons = (outside 10, middle 15, innerside 7) (multi)

a. 300 - 1 - 33 = 266 (auto) a. 300 - 1 - 10 = 289 (multi)
b. 337 + 60 - 33 = 364 (auto) b. 337 + 32 - 10 = 359(multi)

* Reelability % = 266/364 x 100 = 73 % (auto)
* Reelability % = 289/359 x 100 = 81 % (multi)

2. Length of cocoon filament (m) = $\frac{\text{length of raw silk X average reeling cocoon No.}}{\text{number of reeling cocoons}}$

   a. 1. Auto-number of reeling cocoons = testing cocoons - number of unreeled conversion cocoons-(number of carry over conversion cocoons + increase or decrease number of cocoons).

   2. Multi-number of reeling cocoons = testing cocoons - number of unreeled conversion cocoons - number of carry over conversion cocoons.

e.g. Testing cocoons = 300 ;
Total length of raw silk = 36,580
Average reeling cocoon number = 9.67 (auto)
Reeling cocoon number = 8.00 (multi)
Reelability = 73 % (auto);
Unreeled cocoons = (fully 1, middle 3);
Carry over cocoons = (outside 26, middle 27, innerside 7) (auto)
Carry over cocoons = (outside 10, middle 15, innerside 7) (multi)

a. 1. 300 - 2 - (33 + 1) = 264 (auto)
2. 300 - 2 - 10 = 288 (multi)

* length of the cocoon filament = 36580 X 9.67/264 = 1,340 (m) - auto
= 36580 X 8.00/288 = 1,016 (m) - multi

3. Weight of cocoon filament (cg) = $\frac{\text{conditioned weight of raw silk}}{\text{number of reeling cocoons}}$

a. Conditioned weight of raw silk = anhydride raw silk weight x 1.11

b. 1. Auto---number of reeling cocoons = testing cocoons - number of unreeled conversion cocoons - (number of carry over conversion cocoons + increase or decrease number of cocoons).

2. Multi---number of reeling cocoons = testing cocoons - number of unreeled conversion cocoons - number of carry over conversion cocoons.

e.g. testing cocoons = 300
conditioned weight = 110.21 g
reelability = 73 %
unreeled cocoons = (fully 1, middle 3)
carry over cocoons = (outside 26, middle 27, innerside 7)(auto)
carry over cocoons = (outside 10, middle 15, innerside 7)(multi)
b. 300 - 2 - (29 + 1) = 268 (auto)
300 - 2 - 7 = 291 (multi)

* weight of cocoon filament = 110.21 /268 = 0.411 g (auto)
= 110.21 /291 = 0.379 g (multi)

4. Size of cocoon filament (d) = $\frac{\text{conditioned weight of raw silk x9000}}{\text{length of raw silk x average reeling cocoon number}}$

e.g. conditioned weight of raw silk = 110.21 g
total length of raw silk = 36,580 m
average reeling cocoon number = 9.67 (auto)
reeling cocoon number = 8.00 (multi)

* size of cocoon filament = (110.21x9000)/(36,580x9.67) = 2.80d (auto)
= (110.21x9000)/(36,580x8.00) = 3.38d (multi)

5. Raw silk percentage (%) = $\frac{\text{conditioned weight of raw silk}}{\text{weight of green cocoon}}$ x 100

a. Weight of green cocoon = weight of green cocoon (single) x number of reeling cocoons

b. 1. Auto-number of reeling cocoon = testing cocoons - number of unreeled conversion cocoons - (number of carry over conversion cocoons + increase or decrease number of cocoons).

2. Multi-number of reeling cocoons = testing cocoons - number of unreeled conversion cocoons - number of carry over conversion cocoons.

e.g. Conditioned weight of raw silk = 110.21 g
Single cocoon weight of green cocoon = 1.95 g
Unreeled cocoons = (fully 1, middle 3);
Carry over cocoons = (outside 26, middle 27, innerside 7)(auto)
Carry over cocoons = (outside 10, middle 15, innerside 7)(multi)

b. 300-2-(29 + 1) = 268 (auto)
300-2-7 = 291 (multi)

* Raw silk percentage = (110.21 /1.95 x 268)x100 = 21.09 % (auto)
=(110.21 /1.95 x 291)x100 = 19.42 % (multi)

9.4. Conversion table

Length (unreeled) = 1.00p + 0.58h + 0.24m + 0.06l	multi end reeling machine
Weight (unreeled) = 1.00p + 0.43h + 0.14m + 0.03l	multi end reeling machine
Length (carry over) = 0.58h + 0.24m + 0.06l	multi end reeling machine
Weight (carry over) = 0.43h + 0.14m + 0.03l	multi end reeling machine

Length = 1.00p + 0.85h + 0.37m + 0.11l	automatic reeling machine
Weight = 1.00p + 0.80h + 0.28m + 0.06l	automatic reeling machine

Automatic reeling machine

Reelability (%)	42 % ~ below	43 % ~ 52 %	53 % ~ 62 %	63 % ~ 72 %	73 % ~ 82 %	83 % ~ above
±	- 4	- 2	- 1	0	+1	+3

9.5. Degumming (Boil-off-loss)

Degumming is a process of removing the sericin or gum from silk yarn or fabric by boiling with soap solution. Degumming is also called "Boil-off-loss".

Boil-off-loss is required to be determined to provide better cohesion and strength to the yarn. If boil off loss is more the strength of the yarn decreases. Gum is required to give a better finish to the multi striated fibrils. Hence, optimum sericin loss with reference to cocoon shell should not exceed more than 24%.

The following procedure is adopted for estimation of Boil- off-loss with reference to cocoon-shell.

- 10 Cocoon-shells are dried at 105°C for 5 hours.
- Keep the cocoon shells in the dessicator for 30 minutes.
- Record total shell weight.
- Put into a bag (hemp/cotton cloth).

- Make a solution with anhydride sodium carbonate 1 g + "marseilles" soap powder 2 g (pure) + Water 1,000 cc.
- Put the bag(s) with cocoon shells into the solution. Heat upto boiling point for 40 mts. (indirect boiling).
- Change the solution.
- Boil again for 40 minutes.
- Wash in 0.05% anhydride sodium carbonate solution.
- Wash in water in washing machine for 15 minutes with low speed.
- Take out the bag.
- Dry it again at 105°C for 5 hours.
- Keep in dessicator for 30 minutes.
- Record the weight.

$$\text{Boil-off-loss} = \frac{\text{Previous weight - After weight}}{\text{Previous weight}} \times 100 = \#\#.\#\%$$

$$\text{e.g.} \quad \frac{4.23 - 3.12}{4.23} \times 100 = 26.24 = 26.2\%$$

10.0. EGG PRODUCTION

The seed production consists of procurement of seed cocoons, sorting, sexing, moth emergence, coupling, decoupling, oviposition and mother moth examination.

The silkworm eggs from which cocoons are raised for the preparation of commercial seed are called parent seed. The commercial seed is a hybrid involving two or more parents and is known as industrial seed.

After processing of each batch and before the arrival of seed cocoons for the next batch, the cocoon preservation rooms along with the equipments should be thoroughly disinfected with 5% bleaching powder or 2-3 % formalin. Soon after receiving the seed cocoons, the egg production centres should observe the following aspects.

- Check/record the quality and quantity of cocoons received.
- Cocoons of each breed and batches of different farmers, lot number are to be labelled systematically and preserved separately.
- Defloss the cocoons (Fig.15) and sort out flimsy, uzi infested, deformed, melted, thin and open end etc., and keep only good cocoons conforming to the breed characteristics for seed production.
- Pack the seed cocoons loosely in perforated boxes or bamboo baskets in small quantities.
- Preserve the cocoons in single layer in the trays.
- Provide good ventilation in the cocoon preservation room.

- Preserve cocoons/pupae at optimum temperature (25°C) and humidity (70%).
- Conduct pupal gut examination immediately after the arrival of seed cocoons for the incidence of pebrine.
- Subject a sample of cocoons from each lot to high temperature of 35°C for early ecloson and to facilitate early detection of pebrine. Test the emerged moths for pebrine disease to eliminate risk and wastage of labour.
- Cut open at one end of the cocoon to remove the pupa for determining the sex.
- Normally sexing is done manually by visual examination of their sexual markings.
- Cut and separate the cocoons at least three days before emergence. It is especially important for races showing above 20% cocoon shell ratio.
- Preserve the seed pupae in trays in single layer on corrugated paper or powdered paddy husk (Fig.16).
- Cover the pupae with perforated news paper one day prior to emergence to facilitate the emerged moths come on the paper and collection of moths become easy. Besides, this helps in absorption of urine, while picking.

10.1. Moth emergence

- Moths emerge 12-13 days (room temperature 24-25°C) from the day of spinning. Emergence starts early in polyvoltines (10-11 days) than bivoltines (12-13 days).
- In case of Pure Mysore, since the cocoon shell layer is soft, sex separation is little difficult (because pupa may be damaged while cutting the cocoon shell). But as the male and female moths emerge at two distinct peaks they are comfortably collected separately.

10.2. Synchronisation of moth emergence

Planning for synchronisation should start at the brushing time of parental breeds.

- If the cocoons are meant for hybrid preparation, then the total incubation, rearing period and emergence of moths are to be calculated and accordingly the dumbbell lines are brushed two days earlier than oval lines, so that male and female moths are readily available for hybridisation. Sometimes there may be difference in the spinning of the two breeds due to the rearing conditions . In such cases, the emergence of moths in the two breeds can be adjusted by selecting cocoons of matching spinning date.
- If the synchronisation is to be done at pupal stage, the pupae should be refrigerated, preferably 1 day prior to emergence, for four to six days (maximum) at 5 to 10°C.

10.3. Coupling

- Keep coupled moths undisturbed for 3-4 hours in illuminated and well ventilated place. Maintain temperature and humidity at 23-25°C and 70-75% respectively.
- Gently tap the decoupled female moths to induce urination.
- Cold store the male moths immediately after separation at 5°C upto 3-4 days .

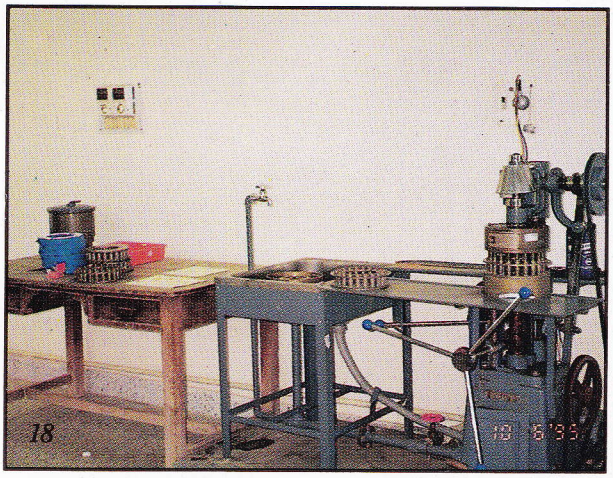
10.4. Oviposition

- Place the mother moths on the egg sheets after urination and in a cellule or composite cellule . Moth cups or cellules are not required for preparation of loose eggs, as the females are allowed to lay eggs on starch paper.
- Provide darkness in the oviposition room, as the light disturb the moth during egg laying.
- Maintain 25°C temperature and 75% relative humidity in the oviposition room.

10.5. Preparation of loose eggs

Silkworm eggs have a gluey coating and adhere to the egg card when laid. If the eggs are laid on starch coated paper, they detach easily when soaked in water. The following procedure is followed for preparation of loose eggs.

- Dissolve about 20 g of arrowroot powder in one litre of water and boil to make a thin paste.
- Smear the paste over the egg sheets as a thin layer using brush or foam strips and dried.
- Allow the moths to lay the eggs on starched sheets/cloth.
- After egg laying, examine the moths and dip the sheets in water for 30-60 minutes.
- After soaking remove the eggs gently from egg sheets under running water to separate the eggs.
- Collect the loosened eggs in a thin muslin bag or perforated plastic container.
- Soak the loosened eggs in 0.5% bleaching powder solution for 5-10 minutes to remove the glutinous substance of the eggs.
- Put the eggs into salt solution (1.095 specific gravity (SG)) with brine assessment tool. The abnormal eggs (unfertilised eggs or dead eggs) will settle down, then take out them into the container.
- Wash the eggs in water to remove the salt solution and dry in shade.
- Disinfect the eggs in 2% formalin solution.
- Wash the eggs in water and spread them on blotting paper for drying.
- In case of bivoltines, collect the eggs in perforated plastic container or double layer muslin cloth and treat them in hydrochloric acid.
- After treatment wash the eggs thoroughly in running water and dry under shade.
- Weigh one gram of eggs and count the number of eggs per gram.
- Find out the weight of 20,000 eggs and pack a unit number of 20,000 eggs in each box.
- Paste a slip indicating race, weight of the eggs, date of laying, probable date of hatching etc.



Figs.16 - 18 Egg Production

Fig.16 Preservation of pupa on corrigated paper
Fig.17 Preservation of dry moth packets
Fig.18 Moth crushing machine (Nagahara type)

10.6. Moth examination

To ensure that the eggs are free from pebrine, the mother moths are subjected for microscopic examination. Moth examination can be conducted by using fresh (green) or dry moths.

- Green moth examination can be conducted when the eggs are meant for immediate use. If the eggs are to be hibernated, the moths are allowed to lay eggs for two days and are tested later.
- The mother moths are systematically placed in dry moth boxes meant for it. While placing the mother moths, the wings are to be properly put into the box, head downwards.
- Before drying, the moth packets/boxes are to be kept safely so that there should not be any damage by ants, lizards etc. Under high humid conditions, misty or powdery mildew type appearance may occur on the body of the moths. So care should be taken while preserving the moths before drying.
- Suppose, the mother moth is dead after oviposition, it has to be tested for disease. The mother moths are dried in hot air oven, at 70°C , for 5 to 6 hours and preserved for later testing in a big plastic box containing silica gel (Fig.17).

Moth examination is of two kinds viz., individual moth examination and mass examination.

10.6.1. Mass examination

This method is suitable for quick and effective inspection of commercial seed. Mother moths are ground with cutter type mixer, sediment is collected by centrifugation. One egg laying sheet is one lot (i.e., 20 moths). If Pebrine is noticed while testing the concerned sheet should be burnt.

- Transfer 20 moths to the crusher together with 80 ml of 0.5% of Potassium Carbonate (K_2CO_3) solution and ground for 2 minutes at 9,000 rpm to separate the spores from the tissue.
- Allow the crushed material to settle for 2 minutes.
- Pour the fluid to the funnels containing cotton layer for filtering and collect the filtered fluid in the tubes.
- Centrifuge the fluid at 3000 rpm for 3 minutes for sedimentation of the spores.
- Reject the supernatant and retain the centrifugal sediment.
- Keep the tube with sediment on a running cyclomixer for proper suspension.
- Draw two drops of smears separately, by using glass rod to the slide and cover the smear with cover glass.
- Observe under the microscope and examine at least 5 fields in each smear.
- For moth testing use magnification of 600 X (objective of 40 x and eye-piece of 15 x) microscope is recommended.

10.6.2. Individual moth examination

In this method, each moth is crushed separately in a ceramic mortar with pestle or in a moth crushing machine (Fig.18). This type of examination, though effective, is laborious and time consuming. It is a must for basic seed multiplication level. For green moths testing, 0.5 % (K_2C0_3) and for dry moths testing use of 2% KOH (1 ml) is necessary. After systematic screening, pool the crushed materials of 20 moths together and crush once again in a mixture by adding 60 ml K_2C0_3 solution (for dry moth use 2% KOH solution for crushing). As in mass examination allow the mixture to settle for two minutes. After centrifugation examine the sediment once again for further confirmation. If pebrine spore is noticed then burn all the layings.

10.7. Egg handling

In India, both polyvoltine and bivoltine eggs are produced. The production and handling techniques are different in these two varieties of silkworm. After moth examination the egg cards must be brushed softly to remove the scales of the moths and then are soaked in 2% formalin solution for 5 minutes, washed in water and dried for surface sterilisation. For hibernating egg sheets before putting in cold storage, they must be brushed softly to remove the scales of the moths.

10.8. Polyvoltine eggs

- Generally, polyvoltine eggs do not undergo diapause. The growth of the embryo is continuous and eggs hatch in 9 - 10 days after egg laying.
- The development of the embryo is comparatively faster than bivoltine eggs.
- For postponement of hatching, the eggs can be cold stored on second day after oviposition for maximum number of twenty days.
- Before refrigeration and taking out from cold storage, the eggs should be kept in intermediate temperature of 15°C for 24 hrs.

11.0. BIVOLTINE EGG

11.1. Control of hatching of bivoltine egg

The bivoltine eggs do not hatch under natural condition in 10 days after egg laying since they enter diapause. However, they can be made to hatch whenever desired. This can be achieved by following various methods of hydrochlorisation or preserving them under different hibernation schedules.

11.2. Egg washing and egg surface disinfection

The disease causing germs are attached to egg and eggsheet because of urination and dust. Hence, washing and surface disinfection are necessary.

- Dip the egg sheets in 2% formalin solution for 5 to 10 minutes.
- Afterwards, wash the egg sheets in running water, and brush the sheets gently to remove the dust and moth scales.

- Keep the egg sheets for drying in the disinfected trays inside the rearing house. Do not dry the sheets under sun or by room heater.
- After drying arrange the layings race wise /strain wise with respect to different seasons.

11.3. Hydrochlorisation

Depending upon the need, the bivoltine eggs can be programmed to hatch between 12-70 days after egg laying by means of immediate acid treatment and acid treatment after chilling.

11.3.1. Immediate acid treatment

Bivoltine eggs can be made to hatch, by the hydrochloric acid treatment carried out at 20 hours after oviposition. This method is called immediate acid treatment. For this treatment there are two methods as detailed below : 1) Hot acid treatment and 2) Cold acid treatment. The immediate acid treatment followed by cold storage can be programmed for hatching between 12 to 30 days after egg laying.

11.3.1a. Hot acid treatment

The hot acid treatment procedure is as follows:

- After 20 hours of egg laying (at 25°C and 80% RH), acid treatment should be done.
- Before acid treatment the egg sheets are dipped in 2% formalin for 5-6 minutes and dried under shade.
- Hydrochloric acid of specific gravity 1.075 (15°C) is heated to a temperature of 46.1°C and the silkworm eggs are immersed in the acid for 5-6 minutes (the time differs with respect to strain/race).The acid strength varies with the temperature under which it is measured and therefore, necessary corrections are to be made.
- The specific gravity of the acid is measured at 15°C.
- The acid should be heated through water media in water bath and the acid should not be heated directly.
- Water bath should be thermostatically controlled to maintain desired temperature without any variation.
- Longer duration of dipping results in burning of eggs and shorter duration sometimes causes poor/irregular hatching.
- The acid container should be sufficiently high and there must be sufficient quantity of acid, so that the egg sheets are completely immersed in acid.
- The acid container should not be overloaded with more number of egg sheets and the they should completely be immersed at a time for uniform exposure of eggs to acid.
- After acid treatment the eggs should be thoroughly washed in running water till the acid traces are completely eliminated.
- The washed eggs should be dried under shade and incubated directly or cold stored.

11.3.1b. Cold acid treatment

Cold acid treatment is conducted at room temperature (25°C). The specific gravity of hydrochloric acid is 1.11 (15°C). The dipping duration ranges between 40 to 90 minutes depending on the room temperature.

Since the climatic condition vary from region to region and season to season, the duration of treatment can be adjusted according to existing room temperature. The dipping duration at different acid temperature is given in Table 8.

Table 8 **Egg dipping duration at different acid temperatures**

Age of the egg	24°C	25°C	27°C	29°C
20-24 hour (at 24°C)	60-90 minutes	60-90 minutes	60-80 minutes	40-50 minutes

11.4 Time of treatment

The eggs are acid treated (hot or cold) within 20-24 hours after egg laying. They should not be treated within 10 hours of laying or after colour change (brownish). The peak egg laying time is 7:00 pm . The age of eggs is counted from this time. The time of treatment of eggs is always corresponding with the temperature during oviposition is indicated in Table 9.

Table 9 **Time of acid treatment of eggs corresponding with oviposition temperature**

Temperature during oviposition (°C)	Period after oviposition (hours)	Minimum period after oviposition (hours)
24	20-30	10
27	20-25	10
29	15-20	10

11.5. Postponement of hatching in acid treated eggs

Acid treated eggs hatch within 12 days . The hatching could be delayed upto 20 days by cold storing (5°C). The treated eggs should be cold stored 18 hours after the acid treatment. The cold stored eggs can be released for incubation on any day within 20 days. Refrigeration beyond 20 days results in poor hatching and should be avoided.

11.6. Postponement of acid treatment

When the acid treatment is to be postponed, the eggs before 20 hours after egg laying are cold stored at 5°C upto 5 days. After release from the cold storage, the eggs should be kept for an hour at 15°C before treating with acid.

11.7. Acid treatment after chilling

The bivoltine eggs can be made to hatch in 40-80 days after egg laying by the acid treatment after chilling.

11.7.1. Hatching in 40 - 45 days (short term chilling)

- After egg laying, the eggs are kept at 25°C for 30-35 hours and then cold stored at 5°C for 30-40 days.
- After completion of 30 days the eggs should be released through intermediate temperature of 15°C for 3 hours.
- Then eggs are shifted to 25°C for 2 - 3 hours and treated in acid of 1.10 specific gravity (at 15°C) at 48°C for 5 to 6 minutes (time mentioned differs for each strain/race).
- Longer the cold storage period, better will be the hatching.
- If eggs are released from the cold storage before 30 days, hatching may be irregular.

11.7.2. Hatching in 45-70 days (ordinary chilling)

- The eggs which are laid in 25°C should be stored at 5°C after 40 - 50 hours of egg laying for 45 to 70 days.
- While releasing and cold storing the eggs should pass an intermediate temperature of 15°C for about 6 hours.
- The method of cold storage and acid treatment are same as short term chilling.
- Wash the eggs thoroughly and dry under shade.
- Avoid cold storing of eggs (short term and ordinary chilling) after the acid treatment.

11.8. Preparation of acid

Commercial grade hydrochloric acid (HCl) can be used. Generally, the specific gravity of the commercial acid is ranging from 1.160-1.180. This acid can be diluted to required concentration by adding water to obtain the required volume of desired specific gravity (SG).

$$\text{Quantity of acid required} = \frac{(\text{Desired SG} - 1.000) \times \text{Quantity of acid to be prepared in ml}}{(\text{Available SG} - 1.000)}$$

To this quantity of acid, water has to be added to make up to the required SG.

For example : Available SG of HCl = 1.180
Desired SG of HCl = 1.075
Quantity to be prepared = 1000 ml

Therefore,

$$\frac{(1.075 - 1) \times 1000}{(1.180 - 1)} = 416 \text{ ml of acid}$$

416 ml of acid is to be added to 584 ml of water to get 1000 ml of 1.075 SG of HCl.

Since heat is liberated when water is added to acid, the acid should be prepared 5-6 hrs earlier to treat the eggs, to enable the acid to cool down. The narrow range hydrometer (1.050 -1.100 for hot acid and 1.050-1.120 for cold acid treatment) should be used to test the acid strength for accurate readings as the SG of acid changes with temperature and is inversely proportional to the temperature. The required SG of the acid at a particular temperature is given in Tables 10 and 11. The strength should be checked and corrected accordingly.

The strength of acid will also change depending upon the frequency of acid treatment. Hence, the SG of the acid should be corrected quite often.

Table 10 **Ready reckoner for SG preparation**

	SG	1.075	1.100	1.110
	Hcl	38 L	50 L	55 L
	H2O	62 L	50 L	45 L

NOTE: Hcl = 35 %; L - litre.

Table 11 **Specific gravity correction of acid according to temperature**

TEMP. °C	TEMP. °F	H.A.T	A.T.C	A.T.R
		1.075	1.100	1.110
20	68	1.0732	1.0977	1.1076
23	73	1.0722	1.0964	1.1062
25	77	1.0715	1.0955	1.1052
27	81	1.0708	1.0946	1.1042
29	84	1.0701	1.0937	1.1033
31	88	1.0691	1.0928	1.1023
34	93	1.0683	1.0915	1.1009
46	115	1.0642	1.0861	1.0952
48	118	1.0635	1.0853	1.0943

Note: H.A.T. - Hot acid treatment; A.T.C. - Acid treatment after chilling
A.T.R. - Acid treatment at room temperature.

12.0. HIBERNATION

In univoltines and bivoltines, the eggs undergo diapause in 40-50 hrs after oviposition at 25°C. The newly laid eggs are yellowish in colour and gradually change to brown and then to dark colour. Such eggs do not hatch unless they are activated by cold temperature. Long term cold storage terminates the diapause and activate the eggs to hatch. Specific schedules are followed for processing the hibernated eggs in cold storage.

The hibernation followed in temperate region like Jammu and Kashmir is different from tropical regions. Different schedules of preservation of bivoltine silkworm eggs followed by Jammu and Kashmir are given below :

12.1. Preservation of spring eggs for next spring rearing

12.1a. JAMMU AREA

Spring season in Jammu is much earlier to Kashmir zone. Silkworm rearing starts in March and hybrid eggs are produced in May when the temperature in Jammu is hot. During this period, the eggs are shifted to cooler areas like Batote where the temperature is 25-26°C. The temperature starts falling gradually in July-August and reach 20°C in August and lower (10-15°C)

in September. The eggs are transferred to cold storage at 5°C in November and kept upto third week of December. From this period upto 15-20th of February a temperature of 2.5°C is maintained in cold storage. The eggs are kept at intermediate temperature of 15°C for few days. Later they are released for incubation and rearing in March.

12.1b. KASHMIR AREA

Spring rearing in Kashmir is conducted in May and eggs are prepared in July-August. Till November the eggs are kept in natural temperature when the temperature falls to 15- 10°C slowly by December, these eggs are shifted to cold storage (5°C). After 50-60 days i.e., by the end of February the eggs are shifted to 2.5°C. This temperature is maintained for 60 days, upto the end of April and eggs are released for incubation either at the end of April or the beginning of May as required.

12.2. Hibernation schedule in tropics

In tropics, the seasons are not demarcated and many rearings are conducted. However, the bivoltine eggs can be hibernated in well maintained cold storage under different schedules (4 months, 6 months and 10 months) to make them hatch between 120 days to 300 days. The schedule comprises three distinct phases viz., aestivation or keeping at 25°C, cold storage at 5°C and 2.5°C and an intermediate phase (10-15°C).

The period of aestivation and the duration of cold storage are related. The relationship between the aestivation at 25°C and cold storage at 5°C to obtain satisfactory hatching is detailed in Table 12.

Table 12 **Appropriate schedules for preserving bivoltine eggs**

Temperature °C	Number of days to be preserved		
	4 months	6 months	10 months
25	10	20	30
20	2	15	50
15	2	10	25
10	3	10	25
5	4	60	60
2.5	90	50	50
15 (intermediate care)	I	I	4.5
	I	I	
2.5	I	I	30-40
	I	I	
Incubation	12	12	12

Each developmental stage (embryonic stage) has different sensitivity to low temperature and can not withstand prolonged refrigeration at one stretch.

When the eggs are preserved for 10 months schedule two step refrigeration has to be followed. After the termination of diapause, the eggs can be allowed to reach "longest embryo" stage which can withstand further cold storage. Thus intermediate care of exposing eggs to 15°C for 4 to 5 days is followed to attain longest embryo stage after which the eggs are again

refrigerated at 2.5°C. Intermediate care is given about 40 to 50 days before the expected date of hatching.

Eggs preserved under different hibernation schedules can be released 12 days early or later than the schedule period without affecting hatchability.

13.0. MULTIPLICATION OF SILKWORM BREEDS AT P4 AND P3 LEVEL

"Seed organisation" comprises the maintenance of breeder's stock and its multiplication for the ultimate production of large quantity of commercial hybrid seed. Therefore, the maintenance of breed characteristics (purity, vigour etc.) is of utmost importance. The breeder's stock maintenance should be the responsibility of breeders of Research Institutes, which in turn (now and then) should supply the basic seed for further multiplication. The breeder's stocks will be multiplied 3-4 times in a year (favourable months) and the different multiplication levels are designated as P3, P2 and P1. However, a three tier system is considered more ideal and efficient which is followed in all sericulturally advanced countries. The breeder's stock and multiplication centres should be under the control of Government agencies and well trained persons.

13.1. Multiplication cycles

- Reduce the cycles of basic seed multiplication from the present 4 cycles to 3 cycles.
- Produce only required quantity of quality cocoons and layings for further supply.
- Stop the present tendency of producing large quantity of cocoons and layings which are mostly not being used.

13.2. Procedure for multiplication of basic stocks at P4 and P3

- Plan and programme the basic seed production based on the requirement of eggs for next level of multiplication. There should be an integrated programme to supply the eggs to various multiplication centres.
- Prepare a flow chart and supply programme for one year in advance for effective monitoring of the production and supply of basic seed.
- Brush 10-30 dfls of each breed in cellular batches (individual laying) depending upon the demand and supply of layings.
- Count all the larvae after third moult and retain till spinning.
- Sort out dead pupae-cocoons, malformed cocoons, stained cocoons, thin shelled cocoons etc., after the cocoon harvest. Live pupae cocoons only are taken for calculation.
- Calculate pupation rate on the basis of live pupae. Live pupa can be known by the sound when the cocoon is gently shaked. Extreme low pupation percentage batches are to be rejected (e.g.: 10% below the average).
- Calculate the pupation rate for the number of larvae brushed/third moult larvae. Deduct the number of uzi infested larvae and muscardine affected if any, from the original number and calculate the pupation rate only for the remaining basic number of larvae.

- Take 50-60 cocoons at random in each batch and sex them. After sex separation, record mass cocoon weight, shell weight and cocoon shell ratio separately for both male and female cocoons. Take the mean of both the sexes in each batch.
- Calculate the mean of cocoon weight, shell weight and shell ratio of all the batches of the breed.
- Select only the batches above average for pupation (in each breed).
- Eliminate the batches which have produced cocoons with undesirable shapes, thin shell cocoons and poor growth performance.
- In case of P4, select the batches scoring above the average for all the three traits viz., cocoon weight, shell weight and shell ratio (minimum 3 batches are to be selected)
- In case of P4, select individual cocoons. This should be done as per the guidance of the breeder.
- Whereas in P3, select the batches above average or nearer to average for the three traits like in P4 (minimum 3 batches are to be selected).
- Do not resort to cocoon selection for P3 batches. Select only the batches based on their merit.
- For every rearing the P3 layings should be obtained from P4 stock.
- The selected batches of each breed both in P4 and P3 are to be interbred/inter-batch crossing for raising next generation (e.g. 1x 3, 2 x 3, 3 x 1).
- Depending on the required number of dfls, keep equal number of male and female pupae for egg production (e.g. to prepare 100 dfls 300 female and 300 male pupae are to be kept). If there is shortage (less) of males, use the same males two times for egg production.
- After egg laying, all the mother moths should be subjected for pebrine test individually.
- Make provision to preserve (P4 and P3) eggs under different hibernation schedules.
- Replace the breeder's stock from breeders once or twice in a year.

13.3. Multiplication of silkworm breeds at P2 stage

- For every rearing, the P2 layings should be obtained from P3 stock.
- Depending upon the requirement of P1 dfls, the P2 dfls rearing should be prepared. To produce 1000 P1 dfls, 6000 pupae are required i.e., 3000 males and 3000 females. The approximate survival rate is 50 % of the total larvae brushed i.e., 12,000 larvae.
- 5-10 dfls are brushed in mass.
- After cocoon harvest, sort out dead pupae-cocoons, malformed cocoons stained cooons, thin shelled cocoons etc. Live pupae-cocoons are only taken for calculation of pupation rate.
- Adopt mass cocoon assessment of 50 males and 50 females in each batch to record cocoon weight, shell weight and shell percentage.

- Record data systematically.
- Cull out undesirable cocoons. Do not resort to any cocoon selection.
- Utilise all good cocoons for P1 laying production.
- After egg laying, all the mother moths should be subjected for pebrine test individually
- Create data base systematically in Basic Seed Farms (BSF).

13.4. Flow chart for multiplication of P4,P3 and P2

P4 and P3 level

↓

Individual laying/cellular brushing

↓

Batch - (bed) wise cocoon harvest

↓

Calculation of Pupation rate

↓

Assessment of 50-60 cocoons(male and female) bed-wise for cocoon weight, shell weight and shell ratio

↓

Calculation of mean values of all the batches of the breed for cocoon weight, shell weight and shell ratio

↓

Selection of batches above average in P4 nearer to average in P3

↓

Culling undesirable cocoons

↓

Cocoon cutting and sexing

↓

Inter batch (bed) crossing

↓

Preparation of P3 layings from P4 and P2 layings from P3

P2 level

↓

Mass rearing of 5-10 dfls

↓

Batch-wise cocoon harvest and assessment

↓

Mixing of good cocoons from all the batches

↓

Preparation of P1 dfls

13.5. Systematic collection of data in basic seed farms

The collection of systematic data on rearing performance of various breeds is very important in Basic Seed Farms. This helps in evaluating the merits of various silkworm breed characters to facilitate selection of batches on merit to provide quality seed for further multiplication.

13.5.1. Database package on rearing performance

In order to standardise the data collection procedures in all the basic seed farms, a software package has been developed. This package may be used to store and retrieve specific data as per the user requirements through query based structure programmes. The procedure to input basic data into the system are given below. The input data are grouped into five major categories. The parameters pertaining to input and derived in each category are given hereunder.

	Input parameters	Derived parameters
1.	Hatching details	
	No. of hatched eggs	No. of eggs/laying
	No. of unhatched eggs	Hatching on total eggs (%)
	No. of unfertilised eggs	Hatching on fertile eggs(%)
	No. of late born larvae	Actual larvae brushed (No.)
2.	Larval weight and duration	
	Larval weight (g)	Total larval duration both in
	Larval duration in terms of	decimals and days and hour units.
	days and hours I - III, IV	Internal facilities provided
	and V instars	for converting hours into days.
3.	Cocoon yield parameters	
	Uzi infested larvae in number	No. of larvae brushed
	Actual yield by number	Yield/10000 larvae in terms
	Actual yield by weight (kg)	of number and weight (kg)
		Basic larvae number Pupation (%)
4.	Cocoon assessment	
	No. of male and female	Single cocoon weight(g)
	cocoons used for assessment	Single shell weight(g)
	Mass cocoon weight (g)	Shell ratio (%) for male
	Mass shell weight (g)	and female and mean over sex
5.	Cocoon harvest report	
	Good cocoons (No.)	Actual yield (No.)
	Double cocoons (No.)	Good cocoons (No.)
	Melted cocoons (No.)	Good cocoons (%)
	Uzi infested cocoons (No.)	Double cocoons (%)
	Cocoons per litre (No.)	

In addition to the generation of individual reports for each category, a consolidated report consisting of all derived parameters from different reports may also be generated.

An example of an hypothetical model of data formats and reports generated on the above lines are given below :

FORMAT - I HATCHING

LOG No.	EGGS/ LAYING (No.)	HAT-CHED EGGS (No.)	UN-HAT-CHED EGGS (No.)	UN-FERTI LISED EGGS (No.)	LATE BORN LARVAE (No.)	ACTUAL LARVAE BRUSHED (No.)	HATCHING %	
							TOTAL EGGS	FERTILISED EGGS
1	438	350	50	38	48	302	79.9	87.5
2	615	520	25	70	100	420	84.6	95.4
3	453	436	15	2	50	386	96.2	96.7
4	405	387	8	10	40	347	95.6	98.0
5	582	527	35	20	55	472	90.5	93.8
6	438	420	10	80	60	360	79.9	87.5
7	615	515	25	70	50	465	84.6	95.4
8	453	436	15	20	45	391	96.2	96.7
9	405	387	8	50	60	327	95.6	98.0
10	582	415	7	60	50	365	90.5	93.8

FORMAT - II LARVAL PERIOD

LOG No.	I-III LP DAYS	IV LP DAYS	V LP DAYS	TOTAL PERIOD DAYS	I-III LP		IV LP		V LP	
					D	H	D	H	D	H
1	12.08	4.79	6.13	23.00	12	2	4	19	6	3
2	13.00	5.75	7.00	25.75	13	0	5	18	7	0
3	11.17	5.42	6.04	22.63	11	4	5	10	6	1
4	12.13	5.58	7.00	24.71	12	3	5	14	7	0
5	11.42	5.08	7.71	24.21	11	10	5	2	7	17
6	12.08	5.79	6.13	24.00	12	2	5	19	6	3
7	11.00	4.75	7.13	22.88	11	0	4	18	7	3
8	12.17	5.42	6.04	23.63	12	4	5	10	6	1
9	11.13	5.58	6.13	22.83	11	3	5	14	6	3
10	12.04	5.71	7.08	24.83	12	1	5	17	7	2

NOTE : Input data is Instar-wise Days and Hours. LP : Larval Period

FORMAT - III COCOON YIELD

LOG No.	ACTUAL BRU-SHED LARVAE	LARVAE AFTER III MOULT	UZI INFES-TED	ACTUAL YIELD BY No.	ACTUAL YIELD BY WT. (kg)	YIELD/ 10000 BY No.	LARVAE BY WT. (kg)
1	302	282	20	250	0.420	8865	14.89
2	420	390	30	350	0.750	8974	19.23
3	386	378	8	300	0.670	7937	17.72
4	347	338	9	325	0.630	9615	18.64
5	472	466	6	400	0.830	8584	17.81
6	360	350	10	320	0.650	9143	18.57
7	465	458	7	435	0.830	9498	19.23
8	391	383	8	356	0.670	9295	17.49
9	327	320	7	280	0.580	8750	18.64
10	365	356	9	325	0.690	9129	17.81

FORMAT - IV COCOON ASSESSMENT

LOG No.	SEX	No.	COCOON WT. (g)	SHELL WT. (g)	CWT. (g)	SWT. (g)	SR (%)
1	F	17	26.6	5.1			
	M	25	31.7	6.6			
	MEAN				1.42	0.282	19.9
2	F	50	104.0	29.0			
	M	50	96.0	23.0			
	MEAN				2.00	0.520	26.0
3	F	50	96.0	26.0			
	M	50	90.0	21.0			
	MEAN				1.86	0.470	25.3
4	F	50	106.0	28.0			
	M	50	98.0	24.0			
	MEAN				1.92	0.489	25.5

- Record data systematically.
- Cull out undesirable cocoons. Do not resort to any cocoon selection.
- Utilise all good cocoons for P1 laying production.
- After egg laying, all the mother moths should be subjected for pebrine test individually
- Create data base systematically in Basic Seed Farms (BSF).

13.4. Flow chart for multiplication of P4,P3 and P2

P4 and P3 level

↓

Individual laying/cellular brushing

↓

Batch - (bed) wise cocoon harvest

↓

Calculation of Pupation rate

↓

Assessment of 50-60 cocoons(male and female) bed-wise for cocoon weight, shell weight and shell ratio

↓

Calculation of mean values of all the batches of the breed for cocoon weight, shell weight and shell ratio

↓

Selection of batches above average in P4 nearer to average in P3

↓

Culling undesirable cocoons

↓

Cocoon cutting and sexing

↓

Inter batch (bed) crossing

↓

Preparation of P3 layings from P4 and P2 layings from P3

P2 level

↓

Mass rearing of 5-10 dfls

↓

Batch-wise cocoon harvest and assessment

↓

Mixing of good cocoons from all the batches

↓

Preparation of P1 dfls

13.5. Systematic collection of data in basic seed farms

The collection of systematic data on rearing performance of various breeds is very important in Basic Seed Farms. This helps in evaluating the merits of various silkworm breed characters to facilitate selection of batches on merit to provide quality seed for further multiplication.

13.5.1. Database package on rearing performance

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	Input parameters	**Derived parameters**
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	No. of unfertilised eggs	Hatching on fertile eggs(%)
	No. of late born larvae	Actual larvae brushed (No.)
2.	Larval weight and duration	
	Larval weight (g)	Total larval duration both in
	Larval duration in terms of	decimals and days and hour units.
	days and hours I - III, IV	Internal facilities provided
	and V instars	for converting hours into days.
3.	Cocoon yield parameters	
	Uzi infested larvae in number	No. of larvae brushed
	Actual yield by number	Yield/10000 larvae in terms
	Actual yield by weight (kg)	of number and weight (kg)
		Basic larvae number Pupation (%)
4.	Cocoon assessment	
	No. of male and female	Single cocoon weight(g)
	cocoons used for assessment	Single shell weight(g)
	Mass cocoon weight (g)	Shell ratio (%) for male
	Mass shell weight (g)	and female and mean over sex
5.	Cocoon harvest report	
	Good cocoons (No.)	Actual yield (No.)
	Double cocoons (No.)	Good cocoons (No.)
	Melted cocoons (No.)	Good cocoons (%)
	Uzi infested cocoons (No.)	Double cocoons (%)
	Cocoons per litre (No.)	

In addition to the generation of individual reports for each category, a consolidated report consisting of all derived parameters from different reports may also be generated.

An example of an hypothetical model of data formats and reports generated on the above lines are given below :

FORMAT - I HATCHING

LOG No.	EGGS/ LAYING (No.)	HAT-CHED EGGS (No.)	UN-HAT-CHED EGGS (No.)	UN-FERTI LISED EGGS (No.)	LATE BORN LARVAE (No.)	ACTUAL LARVAE BRUSHED (No.)	HATCHING %	
							TOTAL EGGS	FERTILISED EGGS
1	438	350	50	38	48	302	79.9	87.5
2	615	520	25	70	100	420	84.6	95.4
3	453	436	15	2	50	386	96.2	96.7
4	405	387	8	10	40	347	95.6	98.0
5	582	527	35	20	55	472	90.5	93.8
6	438	420	10	80	60	360	79.9	87.5
7	615	515	25	70	50	465	84.6	95.4
8	453	436	15	20	45	391	96.2	96.7
9	405	387	8	50	60	327	95.6	98.0
10	582	415	7	60	50	365	90.5	93.8

FORMAT - II LARVAL PERIOD

LOG No.	I-III LP DAYS	IV LP DAYS	V LP DAYS	TOTAL PERIOD DAYS	I-III LP		IV LP		V LP	
					D	H	D	H	D	H
1	12.08	4.79	6.13	23.00	12	2	4	19	6	3
2	13.00	5.75	7.00	25.75	13	0	5	18	7	0
3	11.17	5.42	6.04	22.63	11	4	5	10	6	1
4	12.13	5.58	7.00	24.71	12	3	5	14	7	0
5	11.42	5.08	7.71	24.21	11	10	5	2	7	17
6	12.08	5.79	6.13	24.00	12	2	5	19	6	3
7	11.00	4.75	7.13	22.88	11	0	4	18	7	3
8	12.17	5.42	6.04	23.63	12	4	5	10	6	1
9	11.13	5.58	6.13	22.83	11	3	5	14	6	3
10	12.04	5.71	7.08	24.83	12	1	5	17	7	2

NOTE : Input data is Instar-wise Days and Hours. LP : Larval Period

FORMAT - III COCOON YIELD

LOG No.	ACTUAL BRU-SHED LARVAE	LARVAE AFTER III MOULT	UZI INFES-TED	ACTUAL YIELD BY No.	ACTUAL YIELD BY WT. (kg)	YIELD/ 10000 BY No.	LARVAE BY WT. (kg)
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2	420	390	30	350	0.750	8974	19.23
3	386	378	8	300	0.670	7937	17.72
4	347	338	9	325	0.630	9615	18.64
5	472	466	6	400	0.830	8584	17.81
6	360	350	10	320	0.650	9143	18.57
7	465	458	7	435	0.830	9498	19.23
8	391	383	8	356	0.670	9295	17.49
9	327	320	7	280	0.580	8750	18.64
10	365	356	9	325	0.690	9129	17.81

FORMAT - IV COCOON ASSESSMENT

LOG No.	SEX	No.	COCOON WT. (g)	SHELL WT. (g)	CWT. (g)	SWT. (g)	SR (%)
1	F	17	26.6	5.1			
	M	25	31.7	6.6			
	MEAN				1.42	0.282	19.9
2	F	50	104.0	29.0			
	M	50	96.0	23.0			
	MEAN				2.00	0.520	26.0
3	F	50	96.0	26.0			
	M	50	90.0	21.0			
	MEAN				1.86	0.470	25.3
4	F	50	106.0	28.0			
	M	50	98.0	24.0			
	MEAN				1.92	0.489	25.5

5	F	50	107.5	25.7			
	M	50	84.0	23.2			
	MEAN				2.04	0.520	25.5
6	F	50	100.0	28.0			
	M	50	95.0	22.0			
	MEAN				1.95	0.500	25.6
7	F	50	104.0	29.0			
	M	50	96.0	23.0			
	MEAN				2.00	0.520	26.0
8	F	50	96.0	26.0			
	M	50	90.0	21.0			
	MEAN				1.86	0.470	25.3
9	F	50	106.0	28.0			
	M	50	98.0	24.0			
	MEAN				2.04	0.520	25.5
10	F	50	97.0	27.0			
	M	50	94.0	24.0			
	MEAN				1.91	0.510	26.7

Note: F- Female, M- Male

FORMAT - V POST COCOON

LOG No.	GOOD COCOON No.	DOUBLE COCOON No.	MELTING COCOON No.	UZI INFES-TED No.	CO-COON/ LITRE No.	ACTUAL YIELD BY No.	TOTAL CO-COON No.	GOOD CO-COON No.	DOU-BLE CO-COON No.
1	190	7	8	50	88	204	212	89.6	6.6
2	202	3	7	5	89	208	215	94.0	2.8
3	350	5	15	8	93	360	375	93.3	2.7
4	245	7	11	7	99	259	270	90.7	5.2
5	203	8	52	6	95	219	271	74.9	5.9
6	189	4	10	9	94	204	212	89.6	6.6
7	230	3	7	7	97	208	215	94.0	2.8
8	186	5	15	6	93	360	375	93.3	2.7
9	220	7	11	6	92	259	270	90.7	5.2
10	250	2	8	6	93	219	271	74.9	5.9

REARING PERFORMANCE OF P4/P3 MONTH SEASON

Race	Log No.	Eggs/ Lay No.	Hatching%		Basic Larvae No.	Larval Period		Wt. of 10 Larvae (g)	Actual Yield by No.	Actual Yield by Wt. (kg)	Yield /10000 Larvae		Cocn/ Litre (No.)	COCOON %		SCWT (g)	SSWT (g)	SR (%)
			Total Eggs	Fertl. Eggs		V Age larval Days	Total Larval Days				By No.	By Wt. (kg)		Good Co-coon	Double Co-coon			
A	1	438	79.9	87.5	282	6.13	23.00	28.0	250	0.420	8865	14.89	75	89.6	6.6	1.42	0.282	19.9
	2	615	84.6	95.4	390	7.00	25.75	39.0	350	0.750	8974	19.23	73	94.0	2.8	2.00	0.520	26.0
	3	453	96.2	96.7	378	6.04	22.63	42.0	300	0.670	7937	17.72	68	93.3	2.7	1.86	0.470	25.3
	4	405	95.6	98.0	338	7.00	24.71	37.0	325	0.630	9615	18.64	80	90.7	5.2	1.92	0.489	25.5
	5	582	90.5	93.8	466	7.71	24.21	52.0	400	0.830	8584	17.81	78	74.9	5.9	2.04	0.520	25.5
	6	438	79.9	87.5	350	6.13	24.00	41.0	320	0.650	9143	18.57	75	89.6	6.6	1.42	0.282	19.9
	7	615	84.6	95.4	458	7.13	22.88	50.0	435	0.830	9498	19.23	73	94.0	2.8	2.00	0.520	26.0
	8	453	96.2	96.7	383	6.04	23.63	42.0	356	0.670	9295	17.49	68	93.3	2.7	1.86	0.470	25.3
	9	405	95.6	98.0	320	6.13	22.83	38.0	280	0.580	8750	18.64	80	90.7	5.2	1.92	0.489	25.5
	10	582	90.5	93.8	356	7.08	24.83	40.0	325	0.690	9129	17.81	78	74.9	5.9	2.04	0.520	25.5
MEAN		499	89.36	94.3	371	6.70	23.83	39.6			8795	17.66	75	88.5	4.6	1.85	0.456	24.4
SD		83.69	6.32	3.66				7.76			546.22	1.49	4.17	6.99	1.6	0.22	0.09	2.3

13.6. Production of P1 cocoon

The production of parent seed cocoons (P1) needed for industrial hybrid seed production is organised under private sector. The seed areas are to be distinct for bivoltine and multivoltine breeds.

Parent seed cocoon production can also be organised through selected seed rearers by the respective egg production centres. In such areas the agency which is responsible for hybrid seed production should involve directly to produce the seed cocoons by adopting a requisite number of seed rearers.

13.7.Points for attention in basic seed farms

- Wash hands and feet by running water (tap water) before entering the rearing house.
- Keep separate set of slippers in young age and late age rearing houses and leaf storage room.
- Entry to the rearing house should be restricted to the workers only. Do not allow any visitors inside.
- Do not allow leaf supplier to enter the rearing house. Leaf baskets should be collected by the rearing staff at the door step.
- Do not touch the worms with hand. Use chop sticks or forceps for picking the worms and spreading of the rearing bed.
- Collect diseased, dead and under sized worms using un-used leaves or old news paper and put into the basin containing formalin.
- Do not throw litter on the floor. Start bed cleaning after spreading the rexin sheet or gunny cloth on the floor. Disinfect the rexin sheet or gunny cloth every day after use.

– Use disinfected cleaning nets for bed cleaning. Keep two sets of nets.

– Apply lime and bleaching powder mixture around the rearing house regularly.

14.0. CHARACTERISTICS OF BASIC STOCKS

KA (Fig.19)

Egg : Newly laid eggs are light yellow in colour. The egg number is ranging from 470 to 650. Chorion colour is light yellow

Larva : Newly hatched larvae are blackish in colour. Larvae are plain, bluish white, robust, and active in feeding. Larval duration 23-24 days.

Cocoon: Plumpy ovals. Sometimes multilayered cocoon shell. Medium to coarse cocoon grains.

Survival: 85-90%
Cocoon weight: 1.7-1.9 g
Cocoon shell weight: 0.31-0.36 g
Cocoon shell ratio: 18-19 %
Filament length: 800-900 m
Neatness : 85-90 points

Moths are powdery white with large wings and take 11 to 12 days to emerge after spinning.

Occurrence of non-hibernating eggs is common and increase in seasons when incubation temperature remains low. For synchronisation purpose it must be noted that hatching to emergence is shorter by about 2 days than NB4D2.

NB4D2 (Fig.20)

Egg : Newly laid eggs are bright yellow in colour. The egg number is ranging from 475 to 550. Chorion colour is deep yellow.

Larva : Newly hatched larvae are deep brown in colour. Larvae are plain, faint bluish and robust. Larval duration 24- 25 days. Yellow moulters occasionally seen in second moult.

Cocoon : Japanese type elongated constricted with round ends. Medium cocoon grains (wrinkles). Compact shell layer.

Survival: 90-95%
Cocoon weight: 1.6-1.9 g
Cocoon shell weight: 0.32-0.40 g
Cocoon shell ratio: 20-21 %
Filament length: 900-950 m
Neatness : 85-90 points

Moths are elongated and active take 13-14 days for emergence after spinning. Cocoon ends are to be cut to facilitate easy emergence.

NB7 (Fig.21)

Egg : Newly laid eggs are yellow in colour. The egg number is ranging from 450 to 500. Chorion colour is light yellow to deep yellow.

Larva : Newly hatched larvae are deep brown in colour. Larvae are plain. Mature worms are bluish with reddish tinge. Larval duration 24-25 days.

Cocoon : Elongated oval. Medium grains with scanty floss.

Survival: 80-85%
Cocoon weight: 1.6-1.8 g
Cocoon shell weight: 0.31-0.36 g
Cocoon shell ratio: 19-20 %
Filament length: 800-950 m
Neatness : 85-90 points

Moths are smaller in size. Sometimes vestigeal and curly wings are noticed. Moths take 11-12 days to emerge after spinning.

NB18 (Fig.22)

Egg : Newly laid eggs are bright yellow in colour. The egg number is ranging from 450 to 550. Chorion colour is light yellow to deep yellow.

Larva : Newly hatched larvae are deep brown in colour. Larvae are plain. Larval duration 25-26 days.

Cocoon : Elongated constricted with round ends. Constriction shallow to deep. Medium grains with scanty floss.

Survival: 80-85%
Cocoon weight: 1.7-1.8 g
Cocoon shell weight: 0.35-0.40 g
Cocoon shell ratio: 21-22 %
Filament length: 950-1000 m
Neatness : 85-90 points

Moths take 12-13 days for emergence after spinning. Cocoon ends are to be cut for better emergence. Hatching to emergence period longer by 1.5-2.0 days than NB7 and should be synchronised accordingly.

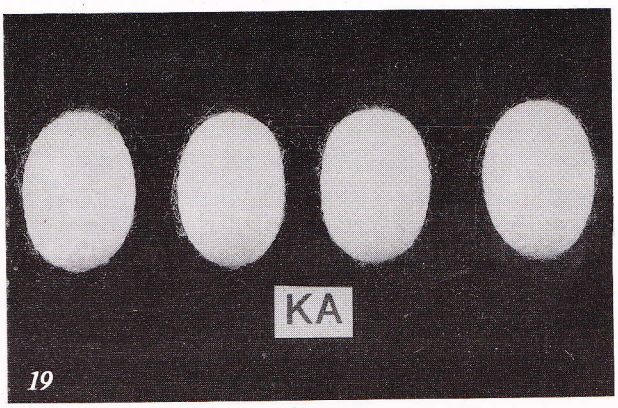
CC1 (Fig.23)

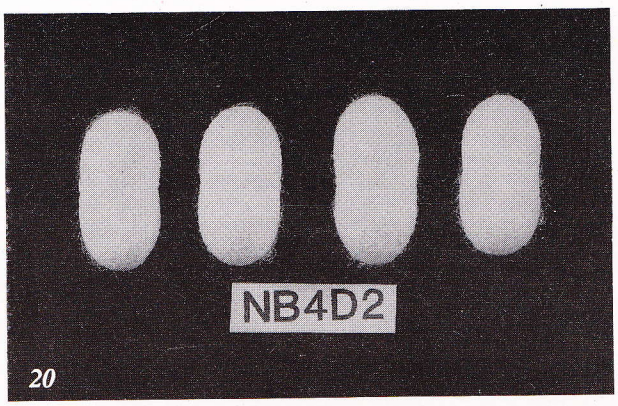
Egg: Newly laid eggs are yellow in colour. The egg number is ranging from 550 to 650. Chorion colour is light yellow to yellow.

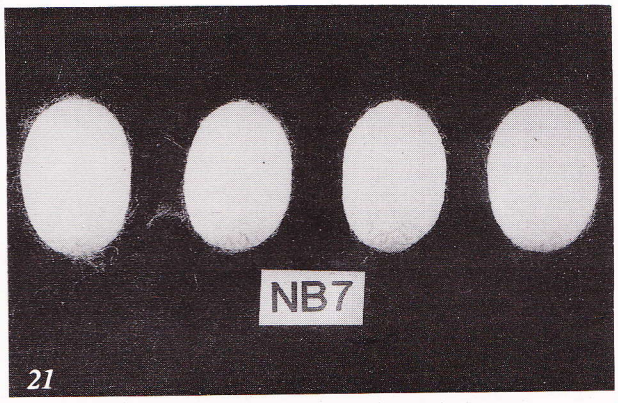
Larva: Newly hatched larvae are deep brown in colour. Larvae are plain, bluish white and plumpy. Larval duration 24-25 days.

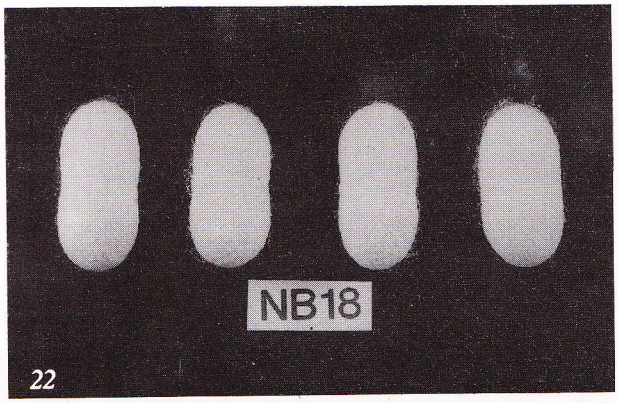
Cocoon: Elongated oval (ellipsoidal) with medium grains.

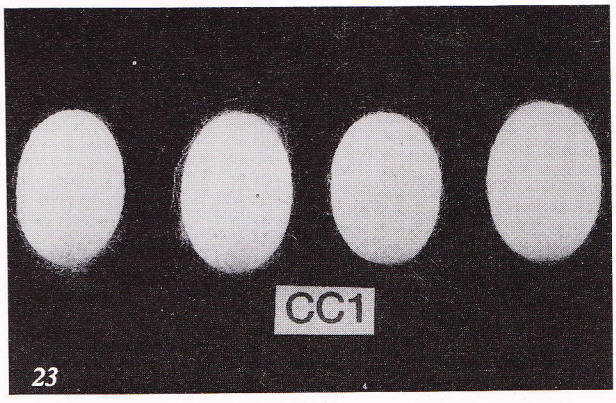
Survival: 90-95%
Cocoon weight: 1.7-2.0 g











Figs.19 - 23 Cocoons of Different Basic Stocks

Fig.19 KA Fig.20 NB_4D_2 Fig.21 NB7 Fig.22 NB18 Fig.23 CC1

Cocoon shell weight: 0.34-0.42 g
Cocoon shell ratio: 20-21 %
Filament length: 900-1050 m
Neatness : 85-90 points

Moths are bigger in size. Cocoon ends are to be cut for easy emergence. Moths take 11-12 days for emergence after spinning.

15.0. MAINTENANCE OF SILKWORM STOCKS (GERMPLASM)

Collection, characterisation, conservation, evaluation and systematic maintenance of silkworm stocks without loosing their original qualitative and quantitative traits are of utmost importance.

15.1. Norms for inclusion

The following categories are generally regarded as important for inclusion in germplasm bank.

1) Geographical races

Include both exotic and indigenous races like, Pure Mysore, Nistari, Sarupat, Moria, C. nichi, Ascoli, Cambodge, C108, Cevanese yellow etc.

2) Breeds in current use

These are breeds of recognised value and selected for commercial use such as KA, NB7, NB18, NB4D2, YS3, JD6, SH6 etc.

3) Breeding materials

- Breeds which are known to be good parents (donor) as breeding materials
- Breeds identified for one or two specific characters or breeds with specific features for eg. survival,shell weight , larval duration , neatness, filament length , CPV and NPV resistant , temperature tolerant etc.,
- Auto sexing breeds: Larval stage sex-limited strains such as N124 quail, sable, zebra, moricaud and egg stage and cocoon colour (WY) stage
- Special genetic stocks : It includes mutants, gene markers, chromosomal variant types such as polyploids ,translocations and deficiency. Parthenogenetically developed clones and moulters are also included in this category
- Old breeds : Breeds which were used in the past, but have now replaced by improved breeds i.e., C108, HS6, NN6D, J122 etc.,

15.2. Criteria and procedure for collection

Centres which are maintaining collections should be advised to rear all the material in one generation preferably in favourable season. Provide full details of quantitative characters with description of special characteristics. The maintaining centre should justify the inclusion of the breed to the accession. The centre should also maintain cocoon or cocoon shell samples. For special features, larvae can also be preserved.

A small committee of 2-3 members from various centres may inspect the data and if necessary, visit and see the samples and decide for inclusion in the accession. Where the committee feels that the materials should be observed during rearing (whose data may be faulty or not clear) before either inclusion or exclusion, the eggs may be obtained and reared in an isolated rearing house and then decide.

15.3. Proforma for collection of silkworm breeds

1) Name of the strain	:	Normal/sex-limited/mutant
2) Date of collection	:	
3) Place of origin	:	
4) Maintained at	:	
5) Year of authorisation/release	:	
6) Breeder's name	:	
7) Code number	:	
8) Geographical or evolved race	:	
9) Parentage	:	
10) Voltinism	:	
11) Fecundity	:	
12) Hatching %	:	
13) Larval pattern	:	
14) Eating behavior	:	
15) V age larval duration	:	
16) Larval duration	:	
17) Cocoon colour	:	
18) Cocoon shape	:	
19) Moulting behavior	:	
20) Cocoon weight	:	
21) Shell weight	:	
22) Shell ratio	:	
23) Filament length*	:	
24) Denier*	:	
25) Neatness*	:	
26) Reelability*	:	
27) Boil-off loss*	:	

** if facilities are available at the centre*

15.4. Description

Descriptive recording is an important aspect which provides the morphological details of each accession enabling to catalogue and classify the material systematically. The germplasm stocks are to be divided into two groups i.e., one stock for biological (basic) research and the other for practical breeding.

Each mutant is described according to the order of developmental stages in the silkworm i.e., egg, larva, pupa, cocoon and adult. While listing mutants, International nomenclature should be used.

15.5. Maintenance

The silkworm eggs of the strains which are received from different breeding centres of India and abroad to the germplasm centre are subjected for surface disinfection with 2% formalin for 5-6 minutes and then washed with water. A sample of eggs are tested under microscope for pebrine disease.

15.6. Rearing Schedule

Multivoltine strains are to be reared 6 times in a year i.e., Feb.-March, April - May , June - July , Aug.-Sept., Oct.- Nov. and Dec.-Jan. The bivoltines are suggested to be reared twice a year in favourable seasons i.e., Jan.-Feb. and July-Aug. by adopting 6 months hibernation schedule. Majority of bivoltine genetic markers/mutants have Diazo genome showing low potency of hibernation. Therefore, such breeds are subjected to 4 months hibernation. Care has to be taken not to store for very long period.

Some genetic markers are sensitive to high temperature (31°C). Therefore, one must take care in providing required quality leaf and environmental conditions.

15.7. Incubation

In order to obtain uniform growth and development of the embryos, which would result in uniform hatching, the eggs are incubated at optimum conditions of temperature and humidity (25°C and 80%).

The incubation room should have temperature and humidity control facility (thermostat) so that any temperature between 18°C and 25°C can be maintained.

15.8. Pattern of brushing

All races/strains of multivoltines and bivoltines are brushed in "composite laying pattern". Composite laying is defined as collection of known number of eggs from a number of 40 individual layings.

15.9. When and how to prepare the composite laying?

40 dfls are taken at random in each race/strain. No selection should be done at laying stage. 40 dfls are divided into 2 batches i.e. 20 dfls in each batch. Both in multivoltine and bivoltine races the composite layings are prepared only after body pigmentation. Approximately 50 eggs from each laying are taken and all the pieces from 20 layings source are pasted on a slightly thick paper and then incubated. Care must be taken that there is no overflow of gum on the eggs while pasting the pieces, because newly hatched larvae may stick on to the gum. Thus each composite laying consists of 20 layings source with about 1,000 individual eggs. Similarly, one more composite laying is prepared from the remaining 20 layings. Thus, in each strain 2 composite layings are prepared for brushing, forming two replications, but each replication is from a different source. At blue egg stage pack the layings in white tissue paper. As a stand by prepare one more composite laying from each race.

For calculation of fecundity 5 individual layings are to be counted separately.

15.10. Brushing

Each composite laying is brushed in one tray . After III moult all the larvae are counted in each bed and 400 larvae (randomly counted) are retained in each replication. Thus each strain will have 800 larvae in two replications coming from 40 layings.

15.11. Special care for multivoltine

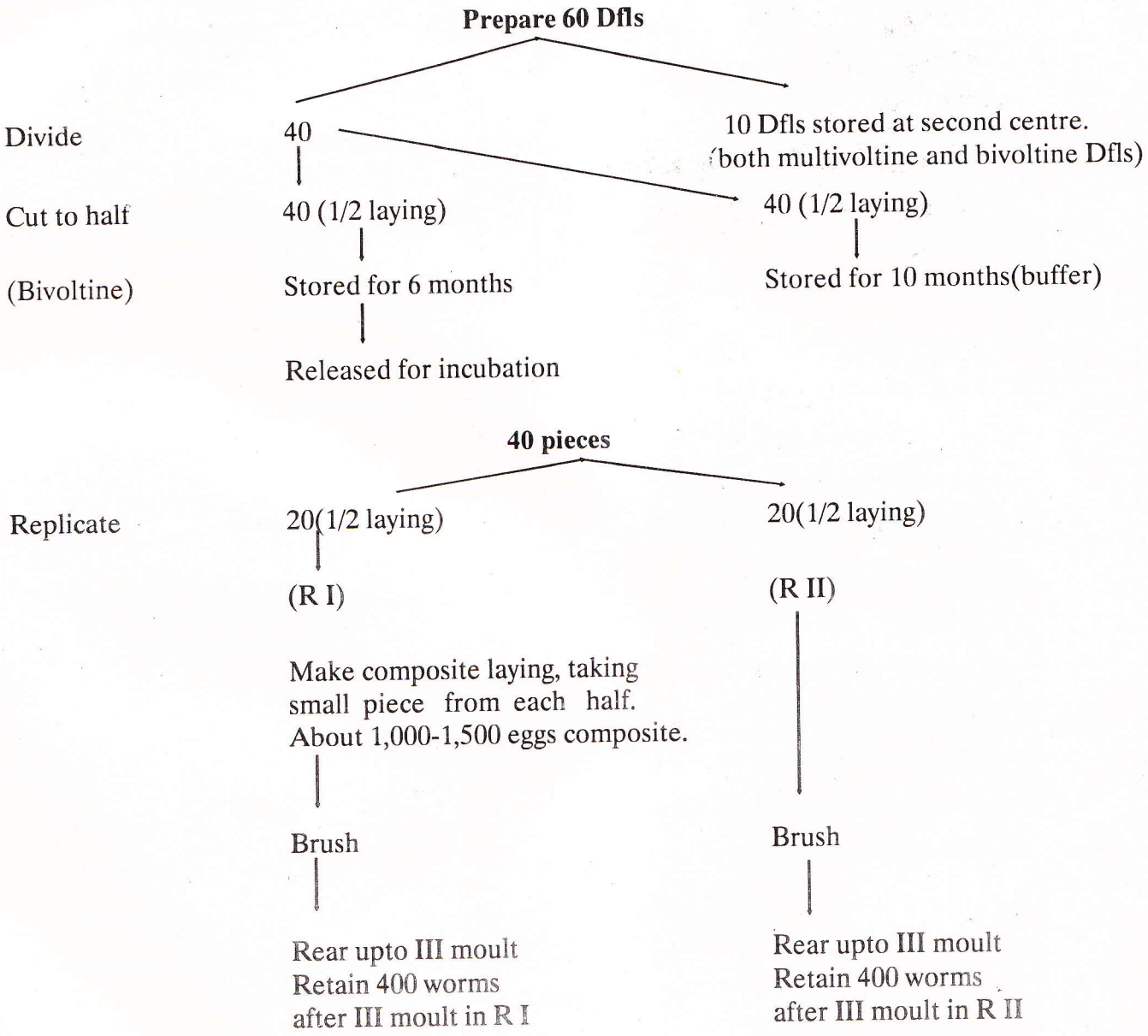
Unlike bivoltines there is no backup available for multivoltine either in cold storage or in another place. Hence, extreme care should be taken while maintaining the multivoltine races. However, the following are also suggested.

- During two rearing seasons (i.e. Oct.- Nov.& Dec.- Jan.) the layings must be incubated at 25-26°C and larvae are reared at 22°C. This may induce hibernating layings to the extent of 20-30% and these layings may be cold stored in the normal method (120-180 days)

– The method suggested by Shimizu (Acta Sericologia Vol.6, pp. 52-59,1973) can also be tried. 30 hrs. old eggs stored at 25°C are shifted to 2.5°C for 30-60 days after intermediate care

– 30 hours old multivoltine eggs are stored at 5°C with optimum humidity beyond 20 days period. (In case of failure of crops, these layings can be immediately provided as the replacement of the failed strain)

15.12. Maintenance norms



The matured worms are collected on time and mounted on the plastic mountages (collapsible). Immediately after mounting all the larvae of one batch, each mountage is covered with nylon net to avoid mixing of larvae from one mountage to another.

15.13. Observations to be recorded

The following observations are to be recorded during egg, larva, cocoon and adult stage.

Egg stage

- Colour of eggs
- Size of egg
- Shape of egg
- Serosa colour
- Chorion colour
- Egg no./1 g on loose eggs
- Eggs/laying (average egg no. about 20 dfls are to be counted)
- Egg laying pattern (periphery or in the centre)
- Voltinism

Larval stage

- Percentage of hatching
- Colour of newly hatched larvae
- Colour of larvae in II moult
- Larval marking
- Larval body colour in V stage
- Total larval duration
- Larval duration (1-3 and 4 instars)
- V age larval duration
- feeding and moulting time at each instar
- Body size (Slender, long, ordinary, short, bulky etc.)
- Larval mortality after IV moult
- Missing percentage in the adult stage

$$= \frac{\text{(Basic larval No.-uzi infested larvae)- Cocooning larvae}}{\text{(Basic larval No.- uzi infested larvae)}} \times 100$$

- Temperature and humidity (1-3, 4, 5 instars and total larval period)
  (If automatic temperature and humidity recorder is available then, calculate the average temperature and humidity once in four hours).
- Uzi infestation (No.)

– Muscardine infection (No.)

Cocoon

– Cocoon colour (bright white, white, cream, green, golden yellow,yellow, pink, flesh etc.)

– Cocoon shape (oval, long or short, elliptical or roundish, ellipsoidal, light constricted, dumbbell, round etc.)

– Cocoon grain (coarse, medium, fine etc.)

– No. of cocoons/litre

– Cocoon weight

– Shell weight

– Shell ratio

– No. of cocoons harvested

– Weight of the cocoons harvested

– No. of cocoons/kg.

– Double cocoons (%)

– Pupation rate (based on basic larval number)

– % of floss (with reference to multivoltine only)

– Stained cocoons

– Open end cocoons

Adult

– Emergence percentage

– Colour of moth

– Egg-laying ratio

For commercial races, the following additional information has to be collected (once in 2 to 3 years)

Denier	Filament length
Reelability	Filament weight
Raw silk percentage/green cocoon weight	Neatness
Degumming loss	Tenacity

In addition to the above, particularly for the breeds which are having economic importance, subjective remarks like activity of the larvae, eating speed, moulting behaviour, spinning duration should also be recorded. Various resistant characters are also be recorded. If needed to do reeling test, a minimum of 100 reelable cocoons are selected and sent for reeling.

15.14. Selection

One day after harvest, double, melted, pointed, etc., are to be sorted out and recorded. Take 100 cocoons (50 male + 50 female) at random for mass weight; then calculate single cocoon weight, single shell weight and shell ratio %. Only bad cocoons are to be rejected and rigid selection should be avoided.

15.15. Egg Production

50 male and 50 female pupae from each replication are placed in cocoon preservation boxes for emergence. Cocoon preservation room should have light and temperature control.

After emergence moths are allowed for random mating. The egg cards are labelled suitably on both sides. The egg sheet and pairs are placed in the same tray. After depairing, the mother moths are kept in a separate oviposition room.

15.16. Utilisation of cocoons

	R I	R II
Worms retained	400	400
*Harvest	300-350	300-350
Reject bad cocoons	-	-
Assessment	50	50
For reeling test	50	-
Cocoons kept for seed	120	120
Layings prepared	30	30
	→ 60 Dfls ←	

(Assessed cocoon shells could be kept as sample and pupae could be used for preparation of layings)

15.17 Formation of committee

A committee has to be formed with the following objectives.

- To evaluate the accessions periodically.
- To reject the duplicates and decide on the new accessions.
- Quantification and classification of breeds for practical breeding. For example to identify and list the breeds with high viability, high fecundity, shorter larval duration, longer filament length etc., this will serve as a guide.
- To characterise the mutant stocks of physical and biochemical aspects.

CONCLUSION

The information/guidelines highlighted with regard to disinfection, incubation, chawki and late age silkworm rearing will help not only in realising the optimum cocoon yield but also help in attaining sustained bivoltine cocoon crops in the field. The silkworm egg handling techniques, hibernation schedules described in the manual are highly useful for Basic Seed Farms and grainuers so as to effect adequate production and timely supply of healthy silkworm seed. The concept and methodology of breed maintenance and multiplication detailed at length can be followed to raise the required healthy seed cocoons conforming to the racial characters for hybrid preparation on commercial scale. The proforma for collection of pure race and hybrid data furnished herein helps in systematic evaluation of the breeds/hybrids at national level. To precise, this manual covering the vital aspects of disinfection, silkworm rearing, cocoon reeling, egg production breed maintenance and multiplication system serves as a necessary tool and a ready reckoner for the extension workers and other technical personnel alike in realising the sustained bivoltine cocoon crops.

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