ORGANISATION OF

Industrial Bivoltine Grainage

FOR TROPICS

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DR. MANJEET S. JOLLY

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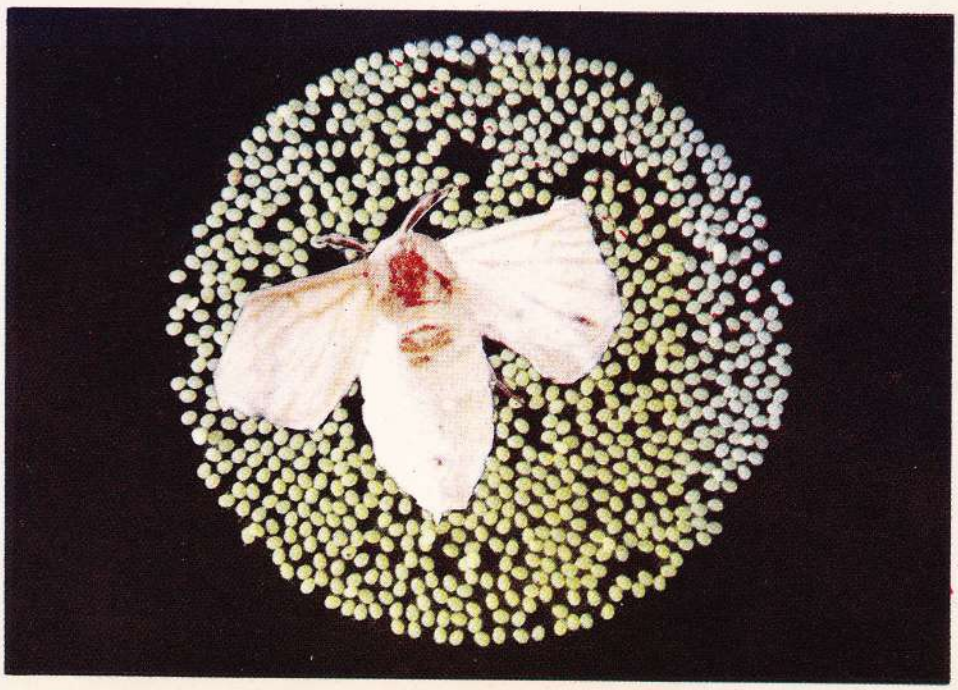

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ORGANISATION OF

INDUSTRIAL BIVOLTINE GRAINAGE

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CONTENTS

I. INTRODUCTION

Quality seed is the backbone of sericulture industry. A proper infrastructure for silkworm seed production is a basic pre-requisite for development of the industry in the country.

The present requirement of silkworm seed in India is estimated at 30 crores disease free layings (Dfls) per annum of which only about 25 crores is being produced in different parts of the country in both Government and private sectors. This production, with the exception of Jammu & Kashmir, Uttar Pradesh and to a small extent Karnataka, is mostly of multivoltine variety and as such, the country's major seed production infrastructure is multivoltine oriented. In view of the vast expansion contemplated for the production of high quality silk in the country, greater emphasis has been laid on the propagation of bivoltine races. The projected target for bivoltine silk production in India, is about 2,000 m.t. by the end of this decade.

The production technology of bivoltine silkworm seed is more complicated than that of multivoltine and unless the produce is processed and preserved properly and made to hatch timely, the above programme will have a set back. The need for proper technology to produce bivoltine seed under the tropical conditions is keenly felt and Central Sericultural Research & Training Institute, Mysore is actively engaged in this direction. The advanced techniques already available in the production of bivoltine seed under tropical conditions need to be adopted on rational lines. This calls for strengthening of the existing silkworm seed production units with necessary technical personnel and equipment.

The present document, in addition to explaining various technical aspects of seed production, will also serve as a guideline on the organisational and the managerial aspects of establishing an Industrial Bivoltine Grainage designed to produce about 25 lakh Dfls (50,000 cases) annually. This could equally serve as a base for tropical countries trying to develop sericulture.

II. WORKING PLAN

The unit of seed handled is a Dfl (Disease free laying) and the number of eggs laid by one moth ranging between 350–500. The seed is also distributed in units of cases and each case contains 20,000 loose silkworm eggs. The eggs sold as units of Dfls are on egg cards. Parental seed is invariably handled on cards.

1. VERTICAL INTEGRATION

The production of industrial seed involves raising of parental seed cocoons' selection, sexing and preservation at optimum conditions and post-emergence processes like coupling, de-coupling, oviposition, inspection for disease and handling of eggs. For better efficiency and quality control, the grainage should be involved in above aspects, viz., from seed crop rearing, distribution of industrial eggs, their hatching etc., and all these operations should be vertically integrated.

A unit of 25 lakh Dfls (50,000 cases) capacity is more ideal as too large an establishment becomes unmanageable, while too small a grainage is uneconomical because of higher investment and larger overheads. The production of 25 lakh Dfls is spread over as per seasonal conditions prevailing in the area. Each month 3 batches with 80,000 layings (1,600 cases)per batch is planned.

Bivoltine seed can be produced throughout the year under the tropical conditions but it is desirable to optimise production during the favourable seasons when the seed crop rearings give higher pupation percentage and better egg yield. The present plan can also be adopted for the sub-tropical areas in India like Jammu & Kashmir, Dehradun (Uttar Pradesh) and Darjeeling (West Bengal) and comparable regions in other countries, where only about 3 crops are harvested in a year. The same plan can be used to produce 25 lakh Dfls in three seasons provided the cocoon preservation facilities are increased. Thus, the grainage will be able to regulate its production according to the requirement with enough built-in-capacity.

2. BASIC FACILITIES

Quality eggs can be produced when the seed cocoons are preserved and processed suitably and the prepared eggs are stored under optimum conditions of temperature and humidity. The critical areas where facilities have to be ensured are: cocoon preservation, oviposition, moth testing and handling of eggs. The present day grainages are deficient in these essential complements and it is imperative that sufficient infrastructural support is provided.

2.1 Cold storage: Cold storage is an essential component of a bivoltine grainage. Domestic refrigerators which are generally used cannot serve the purpose of a cold storage as they do not maintain the required temperature accurately. The advantage of a bivoltine seed is that, it can be preserved and supplied whenever there is a demand. This will not be possible without the support of a cold storage where the temperature could be maintained at 2.5°C, 5°C, 15°C, 20°C and 25°C required for ideal preservation of seed for shorter and longer durations.

2.2 Temperature & humidity: The cocoons are required to be preserved at 25°C to maximise the egg production. Cocoon preservation beyond 32°C results in high percentage of melting and unfertilized eggs, as high temperature induces male sterility. Low temperature of 20°C or below also increases unfertilized eggs. Similarly, proper facility is required during oviposition and post-oviposition period to maintain an optimum temperature of 24–25°C. Fluctuating temperature of 20–35°C in different seasons during aestivation ultimately leads to poor hatching and weak larvae. Experiments undertaken on this line have also confirmed that high temperature and high humidity during aestivation leads to crop failures. As such, an ideal bivoltine grainage should maintain a constant temperature of 25°C and 75–80% relative humidity.

2.3. Grainage building: The grainage building should have adequate facilities for accommodating the cocoons/pupae of different races and sexes in separate rooms. Rooms for coupling and oviposition should be separate. Moth examination laboratory as well as the seed processing sections should be isolated. The incubation

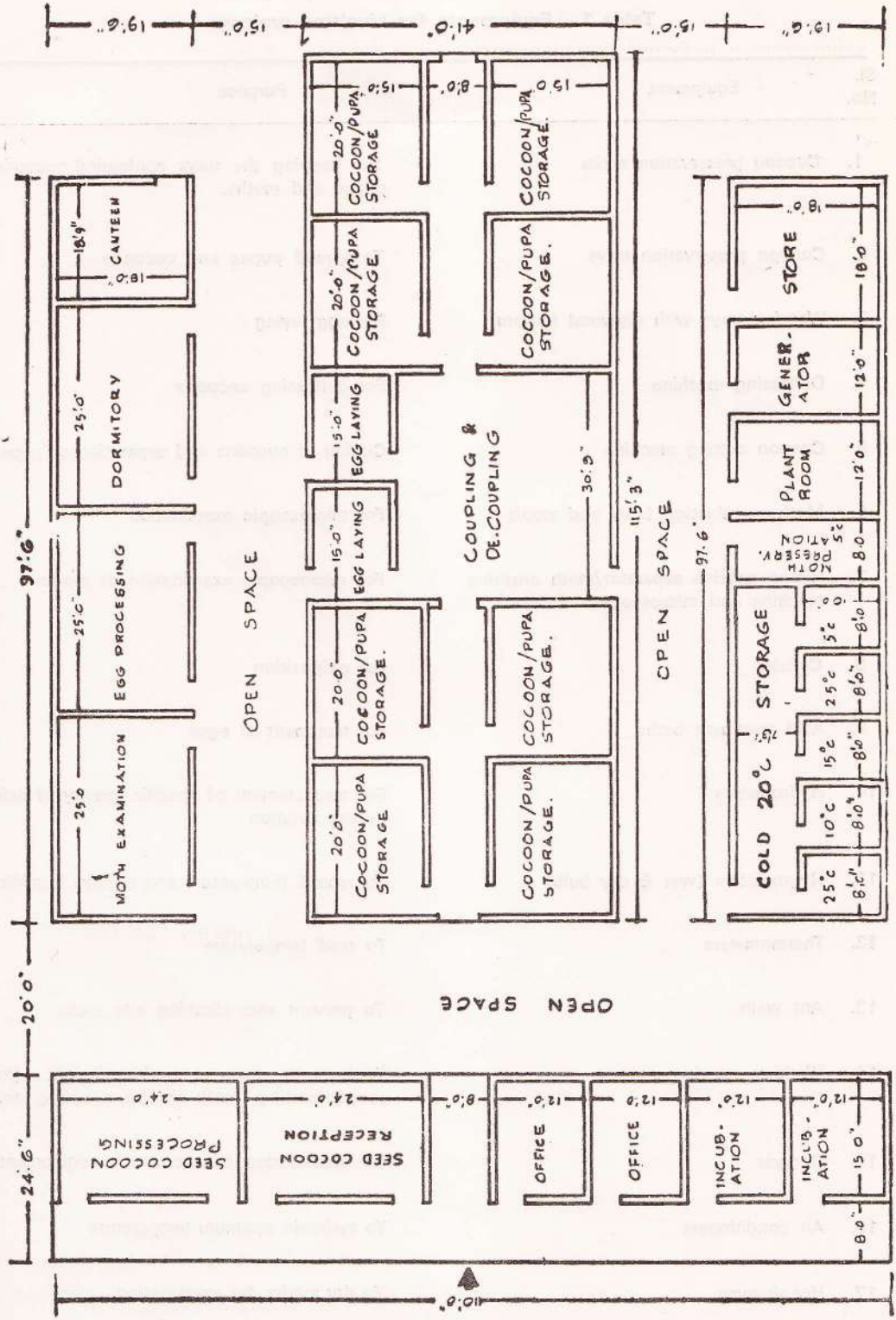


Fig. 1. Plan of grainage building

Table 1. Equipments for bivoltine grainage

Sl. No.	Equipment	Purpose
1.	Cocoon preservation racks	For keeping the trays containing cocoons, pupae and moths.
2.	Cocoon preservation trays	To spread pupae and cocoons
3.	Wooden trays with plywood bottom	For egg laying
4.	Deflossing machine	For deflossing cocoons
5.	Cocoon cutting machine	Cutting of cocoons and separation of pupae
6.	Moth examination table and stools	For microscopic examination
7.	Hirano pebrine separator/moth crushing machine and microscopes	For microscopic examination of moths
8.	Cellules	For oviposition
9.	Acid treatment baths	For treatment of eggs
10.	Hydrometers	For measurement of specific gravity of acid or salt solution
11.	Hygrometers (wet & dry bulb)	To record temperature and relative humidity
12.	Thermometers	To read temperature
13.	Ant wells	To prevent ants climbing into racks
14.	Working stands	To keep the trays at working height (for cocoon sorting, moth picking, coupling etc.)
15.	Sprayer	For disinfection of rooms and equipments
16.	Air conditioners	To maintain optimum temperature
17.	Hot air oven	To dry moths, for examination

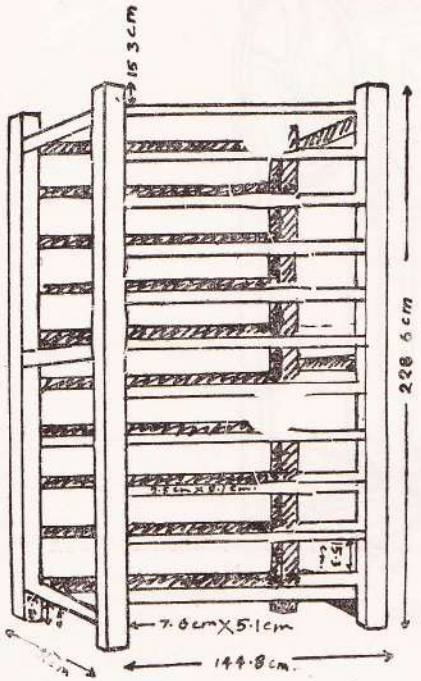


Fig. 2. Cocoon preservation rack

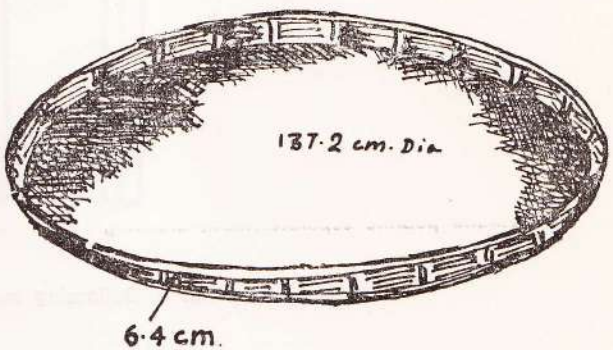


Fig. 3. Cocoon preservation tray

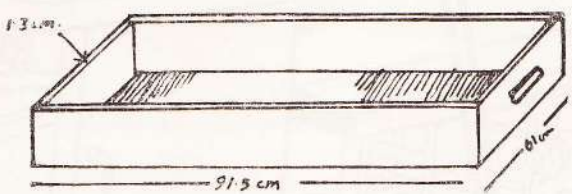


Fig. 4. Wooden tray

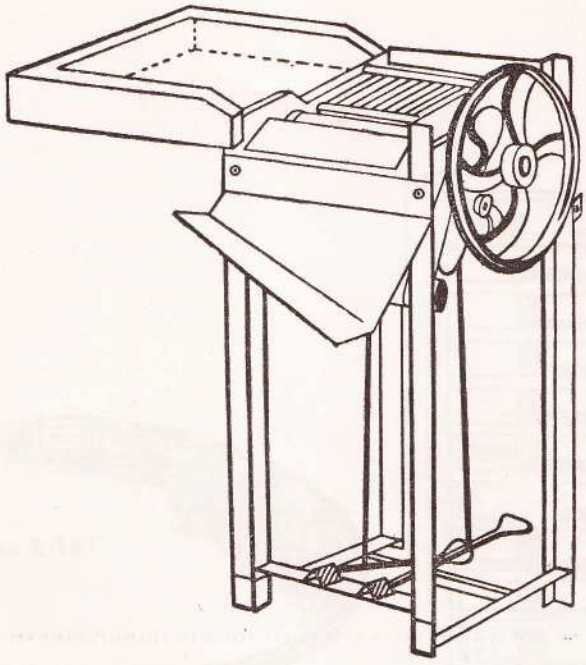


Fig. 5. Deflossing machine

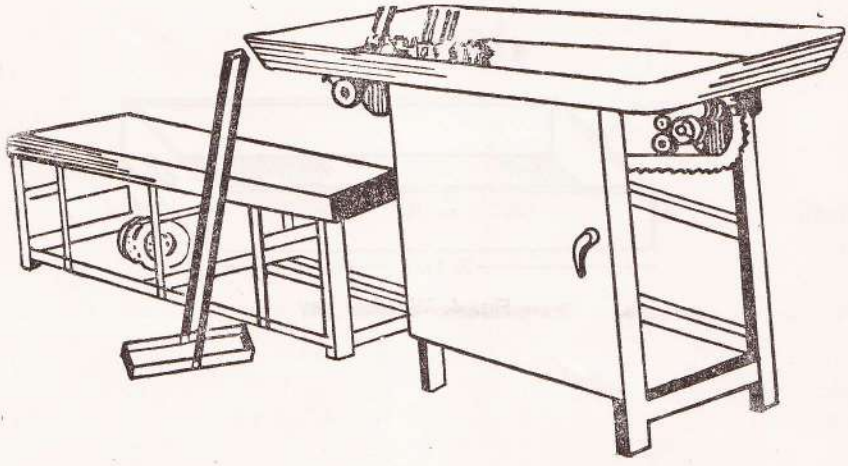


Fig. 6. Cocoon cutting machine

room should be away from seed production wing. A full scale cold storage must be included in the grainage with facilities for storing moths and eggs separately. The provision of a dormitory will increase the efficiency of workers. A plan of grainage building is given in Fig. 1 which will serve as a model design. The dimensions could be altered according to the needs.

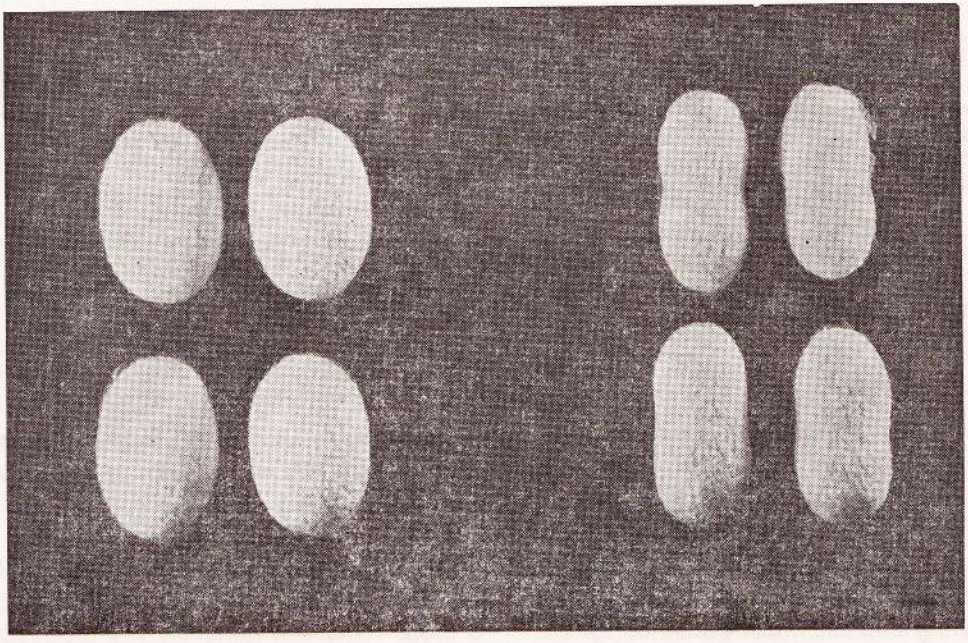
2.4. Equipments: The equipments required for the grainage and their utility are given in Table 1. Essential equipments are racks, round trays, rectangular wooden trays with plywood bottom, antwells, working stands, deflossing and cocoon cutting machines, air-conditioners, moth crushing machines, furniture, microscopes, acid treatment baths etc. The quantity required and their approximate cost are given in Annex 2.

III. TECHNOLOGY OF BIVOLTINE SEED PRODUCTION

1. PRODUCTION OF SEED COCOONS

1.1. Parent seed: 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 referred to as industrial seed.

Two types of silkworm races are used for the production of industrial bivoltine seed viz., Chinese type with oval cocoons and Japanese type with dumb-bell or peanut shaped cocoons (Fig. 7). The parent races must be reared and harvested separately. The seed cocoons of component races are raised in equal quantities for



A. Chinese cocoons B. Japanese cocoons

Fig. 7.

hybrid seed preparation. The parent seed may be a pure race or a hybrid of two races of the same type called foundation cross. The industrial seed produced from such combination of foundation crosses is a double hybrid. Recent researches have confirmed that double hybrids are superior and better for industrial grainage as the egg yield is higher.

1.2. Seed crop rearing: The duration of larval and cocoon stages varies in different races. For the preparation of industrial seed, the emergence of moths in the component races is to be synchronised. The brushing of these races should be programmed accordingly. For example, if one race takes two days more from hatching to eclosion, it must be brushed two days earlier.

The parent seed must be incubated properly at 25°C and 75 to 80% RH, as incubation at lower temperature results in the production of non-hibernating eggs. High temperature incubation leads to poor hatching and weaker larvae. Thorough disinfection of room and appliances should be ensured, before starting the incubation and rearing.

1.2.1. Schedule of rearing: The rearing of seed crop needs more skill than the rearing of industrial hybrid. The parental races are less resistant, less vigorous, have a longer larval duration and poor appetite.

Young age rearings of the seed crop should be done under optimum conditions as the success of the crop depends on the care taken during this stage. The worms in 5th age should be adequately fed with quality leaves; over feeding should be avoided. Schedule for seed crop rearing is detailed in Table 2.

Table 2. Rearing schedule for seed crops for 100 dfls.

Age of Silk-worm	Tempera-ture °C	Humidity %	Size of leaves (cm)	Total qty. of leaf (kg)	No. of feeds/ day	No. of cleanings/ instar	Spacing (rearing seat for 100 dfls sq. ft)
I	27	80-90	0.5 to 2.0	2.5 to 3	3 to 4	1	4 to 14
II	27	80-90	2.0 to 4.0	8 to 9	3 to 4	2	15 to 45
III	26	80	4.0 to 6.0	35 to 45	4 to 5	3	45 to 90
IV	23-25	70-75	Entire leaf	105 to 125	4 to 5	daily	90 to 180
V	23-25	70	Entire leaf	700 to 725	4 to 5	daily	180 to 360

During spinning the temperature should not be high as 32°C or above causes male sterility leading to production of unfertilized eggs and also affects the voltinism. The spinning worms on mountages should not be exposed to direct sun or hot breeze.

1.2.2. Role of grainage: A healthy seed crop is a pre-requisite for producing quality industrial eggs. The grainage should arrange for proper incubation, rearing and technical guidance for the seed crop. This will minimise the production loss of the grainage and help in the smooth programming of production. It is prudent that important operations of incubation and chawki rearing of the seed crop are or-

ganised by the grainage. It would help if the grainage organises co-operative young worm rearing centres exclusively for rearing the parent seed as farmers individually have no facilities to provide conditions required during this stage.

The grainage should have trained extension workers to guide the rearers to harvest healthy and successful crops. The extension agent shall watch the crop carefully and test the larvae and pupae at various stages to make sure that the seed crop is free of pebrine. The report of the extension workers will guide the grainage in obtaining healthy seed cocoons.

1.3. Procurement of seed cocoons: The basic criteria for procurement of seed cocoons are that it should be pebrine free and conform to the norms especially regarding the pupation rate. The norms are fixed according to the season for characters such as cocoon weight, pupation rate and racial characters.

The seed cocoons should be harvested a day later than the industrial cocoons. They can be harvested six days after completion of spinning in hotter areas and seven days in cooler zones. After harvesting, a preliminary sorting is necessary to eliminate flimsy and poor cocoons. Double cocoons, if any, can be retained for seed production.

The seed cocoons are packed loosely in perforated boxes or bamboo baskets in small quantities and are transported during cooler hours of the day. If the live cocoons are tightly packed, they get heated up due to respiration. Exposure to hot sun results in dead pupae and poor emergence.

2. EGG PRODUCTION

2.1. Preparation of grainage: The grainage rooms along with the equipment should be thoroughly disinfected and kept ready before the arrival of seed cocoons. Depending upon the production planned, necessary consumables and the man-power should be kept handy.

2.2. Examination of seed cocoons: Soon after the arrival of seed cocoons, a few pupae from each batch are crushed with a drop of potassium hydroxide and tested microscopically for the incidence of pebrine. As the pebrine spores tend to concentrate in the gut region, it is preferable to remove the gut from the pupae.

In order to facilitate early detection of pebrine, a sample of cocoons from each lot is subjected to high temperature of 35°C for early eclosion. The emerged moths are tested for pebrine disease to eliminate the risk and wastage of labour.

2.3. Sorting: The defective cocoons like flimsy, uzifly infested and deformed are removed (Fig. 8). The good cocoons conforming to the racial characters of the race are preserved. The expected date of emergence is forecast taking into account the pupal development, spinning date and the racial character. This will assist in taking steps to synchronise the emergence of moths of both the parents. Cocoons received

from different seed rearers should be preserved separately to facilitate elimination of diseased lots.

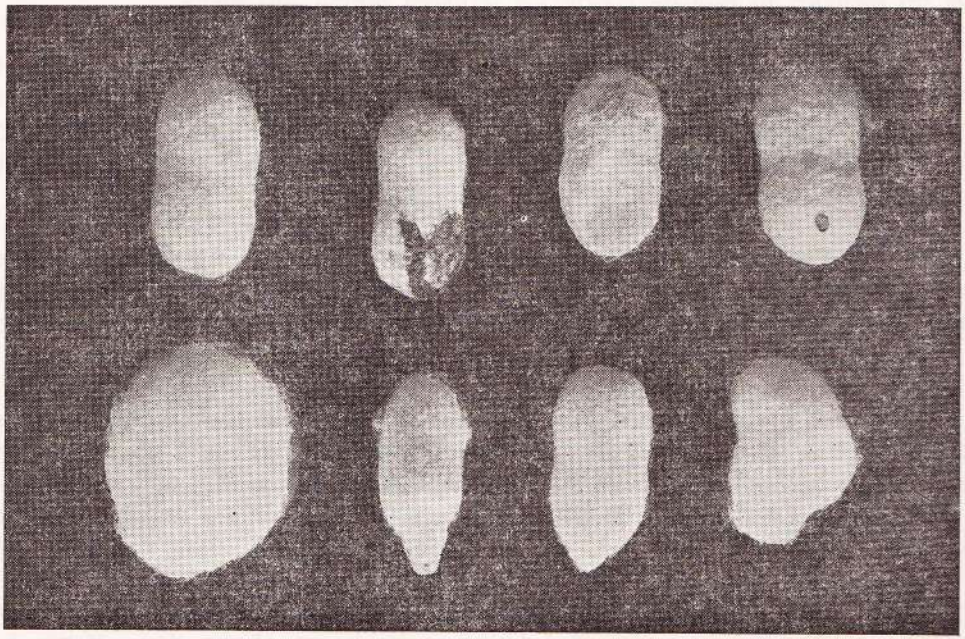
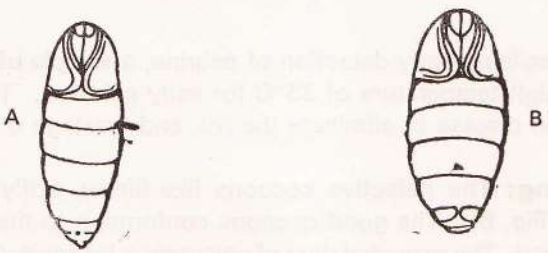


Fig. 8. Defective cocoons

2.4. Cocoon cutting and sexing: The cocoons are deflossed and cut at one end to remove the pupa for determining the sex. Male and female are separated in the pupal stage, based on the marking as illustrated in Fig. 9. Females are larger with broader abdomen and with a '×' marking on the 8th abdominal segment. Males are smaller in size with narrow and pointed abdomen and with a dot like structure on the 8th abdominal segment.

The cocoons should be cut, the pupae sexed and separated at least three days before the expected day of emergence. A skilled worker can separate 12,000-15,000 pupae per day of 8 hours.

In some cases, the cocoons are not sexed and male and female moths are



A. Male pupa B. Female pupa

Fig. 9.

picked up as and when they emerge. This however, results in selfing and affects the quality of seed. Besides, it is cumbersome as workers are to be vigilant all through the emergence period starting from 3 or 4 a.m. Sexing the pupae is a much better and efficient method as it assures true hybrid preparation. The cocoon cutting machine may be used to cut upto 200 kg of cocoon per day. Handcutting is laborious and time consuming. In races with sex-limited larval marking, the male and female can be separated in the larval stage (5th stage). In such cases, the cocoons are to be cut only to facilitate emergence.

Usually cocoons are cut immediately after arrival in the grainage and there may not be any need for preserving the cocoons for more than 1-2 days. In such cases, the cocoons are spread in single layers and preserved in rooms maintained at 24-26°C temperature and 75-80% humidity.

2.5. Preservation of pupae: The sexed pupae are preserved in trays in single layers on corrugated paper or powdered paddy husk. The pupae should not be crowded. Newspaper with perforations of about 1.5 cm in diameter are used to cover the pupae. During emergence, the moths come on the paper and the collection of moths becomes easy.

Males and females of component races should be preserved in separate rooms. It is very important that the temperature of 24-26°C and a relative humidity of 75-80% is maintained in the pupal preservation rooms. Extreme temperatures of 30°C and above or 20°C and below are harmful. The former causes male sterility and unfertilised eggs while the latter, dead and non-hibernating eggs. It is advisable to install air-conditioner in high temperature areas to maintain the desired temperature.

2.6. Synchronisation: Moths of the component races are made to emerge on the same day so that male and female moths are readily available for hybridisation. This is referred to as synchronisation. Planning for synchronisation should start at the brushing time of parent races. However, there may be some difference in spinning due to rearing conditions of the farmers. In such cases, emergence of moths in the two races may be adjusted by selecting cocoons of matching spinning date. In case synchronising batches are not available, emergence of the earlier batches can be delayed by refrigerating the cocoons. The pupae should be refrigerated preferably on the 7th or 8th day after spinning-i. e., 4-6 days after pupation at 5-10°C. Such refrigeration should be limited to 3 days for females and 7 days for males.

The emerged moths can also be refrigerated for the same purpose. The moths may be refrigerated at 5°C upto 10 days in case of males and 2-3 days in case of females. The refrigeration of females either in the pupal or moth stage should be done only as a last resort. Refrigeration should be restricted to any one stage, either in the pupal or moth stage. It is better to preserve the male pupae at 2°C higher than the female pupae by raising the room temperature. Thus, the male moths which emerge earlier, can be refrigerated safely.

2.7. Moth emergence: Moths emerge 12-14 days after spinning.

The cocoon/pupa preservation rooms should be kept dark a day before emergence and bright light is switched on in the early morning by about 5 or 6 a.m., on the day of emergence. Each day's emergence will be over by about 8 a.m. The emergence of each lot generally extends over 3-5 days. After collecting all the moths, the room should be kept dark again until next day morning. The males generally emerge earlier and should be picked up and kept in separate trays for mating purpose.

The moths that have paired (selfed) due to error in sex separation, as also the deformed and weak moths should be rejected while picking (Fig. 10). The healthy moths can be collected from the paper cover easily. If excess males are found, they can be stored at 5°C for later use. The excess females can also be stored likewise, however, it is not desirable.

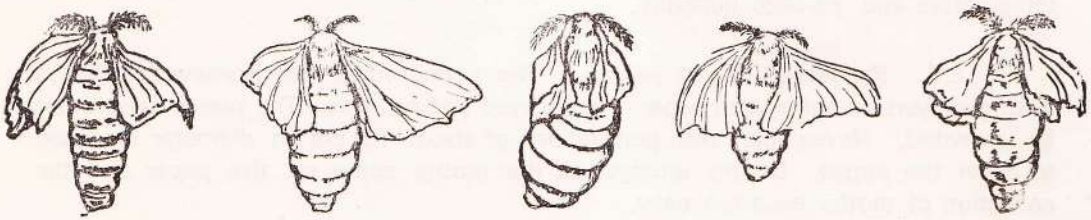


Fig. 10. Deformed moths

The male and female moths of different races can be sprayed with edible colours for easy identification of different components for hybridization.

2.8. Coupling: The female moths are kept in a tray and the male moths of the desired hybrid component are evenly distributed over the females. In about 15 minutes the male and female moths pair. The stray males and the unpaired females are picked up and allowed to mate in a separate tray. The excess males are collected for cold storage. Coupled moths are left undisturbed for 4-5 hours under normal day-light conditions. The temperature and humidity should be maintained at 23-25°C and 75% respectively.

After the required period of coupling the pairs are separated by holding the female moth and gently sliding the male. Violent separation may injure the female copulatory organs. The tray containing decoupled female moths may be gently tapped to induce urination.

Although 4 hours of mating is sufficient, it is desirable that the coupling period is extended to 6-8 hours to get maximum number of eggs laid in shortest period.

2.9. Reuse of male moths: The male moths immediately after separation can be cold stored at 5°C upto 3-4 days for reuse. They have to be taken out of the cold storage half-an-hour before mating for activation.

2.10. Oviposition: When eggs are required to be laid on cards, the mated females are placed on the egg sheets and each moth is enclosed in a cellule. Cellules are not required for preparation of loose eggs, in which case, a unit number

of female moths are allowed to lay eggs on starched paper or cloth, within a wooden or plastic frame. The number of moths vary from 30 to 200 according to the health of the batch and convenience. Moths are allowed to lay eggs for 24 hours in a dark room where the temperature and humidity are maintained at 25°C and 75-80% RH respectively. Since one day old eggs require sufficient oxygen, they should never be kept in small boxes or in closed rooms. The eggs should not be disturbed on the first day.

Cellules are conical in shape and are usually made of black PVC as shown in Fig. 11. The inner surface of the cellule should be smooth to prevent clinging of moths. Egg cards are designed to accommodate 20 or 28 moths, serially numbered. The cellules isolate the eggs laid by each moth, facilitate individual moth examination and elimination of eggs laid by diseased moth. Composite cellules with 20 or 28 rings in one frame can also be used to save labour (Fig. 12).

3. MOTH EXAMINATION

Pebrine is a serious disease which is transmitted to the eggs from the mother moths. To ensure that the eggs are free from pebrine, the female moths are microscopically examined. If the eggs are meant for immediate use and are to be acid treated, the female moths are tested on the 2nd day of egg laying. If the eggs are to be hibernated, the moths are allowed to lay eggs for 2 days and are tested at leisure. In such a a case, the moths are dried at about 75°C for 4-5 hours and preserved for later testing. Drying makes the testing easier, prevents multiplication of secondary pathogens and kills the pebrine spores. Drying the moths at temperature beyond 80°C disfigures the spores.

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

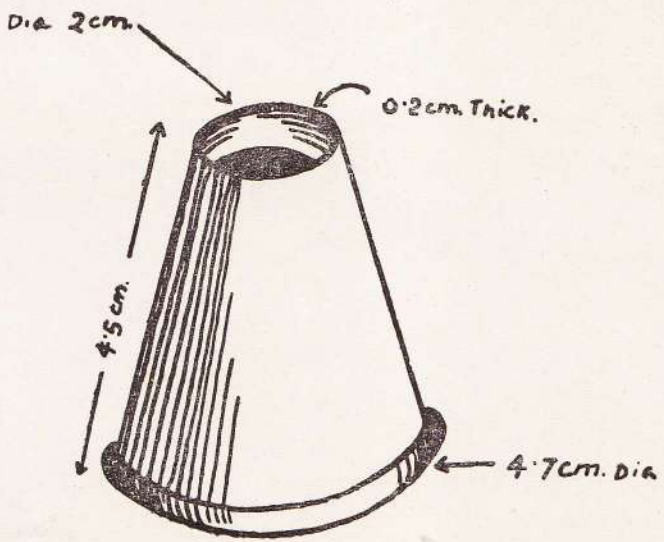


Fig. 11. Cellule

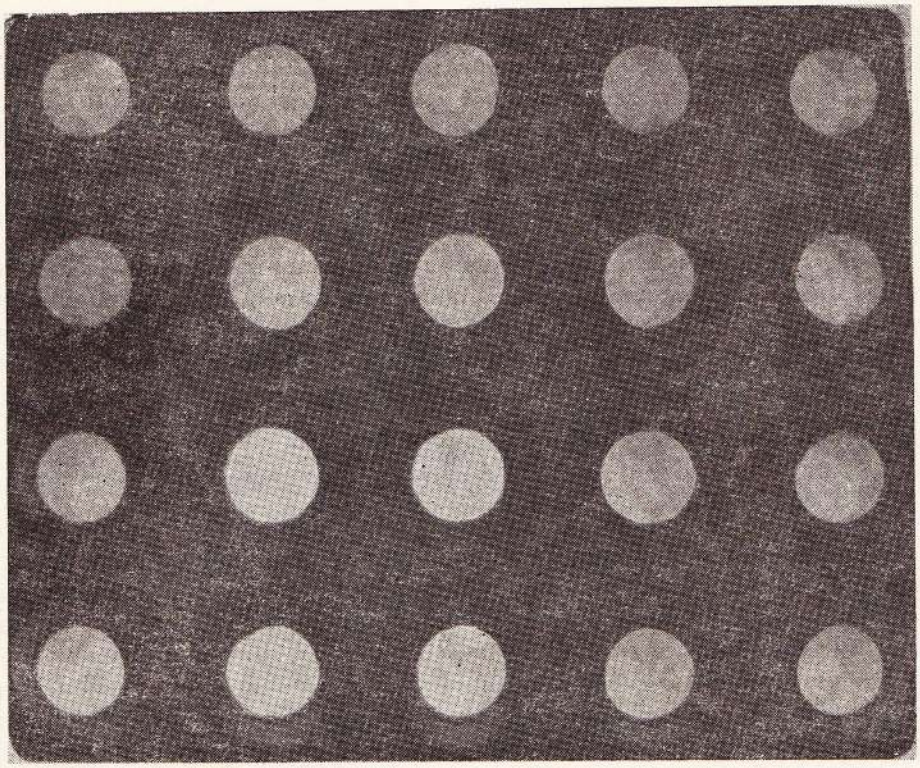


Fig. 12. Composite cellule

3.1. Individual moth examination: This type of testing is conducted for reproductive seeds or for industrial eggs, laid on cards. Here the individual moths

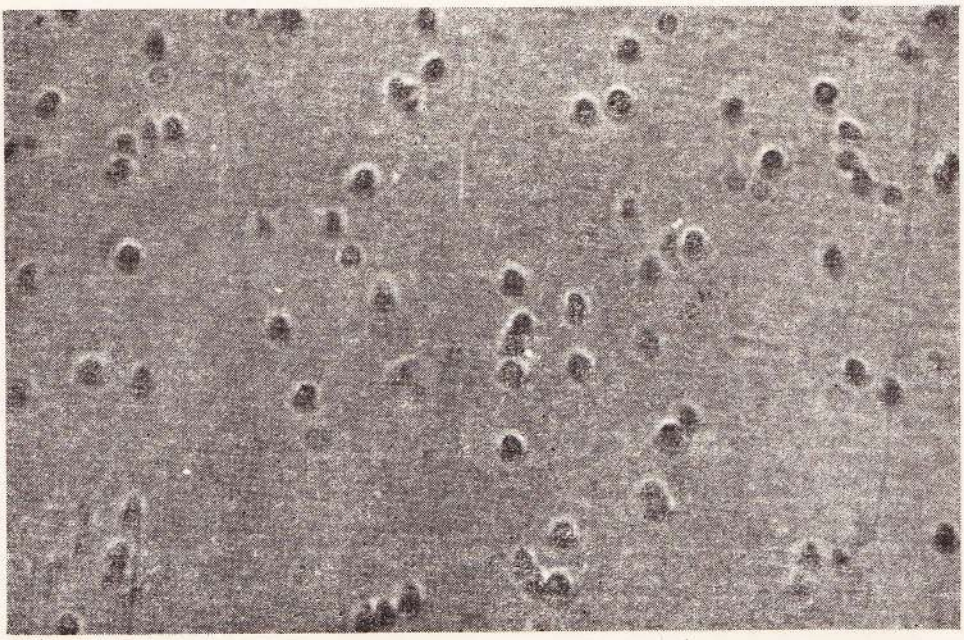


Fig. 13. Microscopic view of pebrine spores

are transferred to the mortars (ceramic or metal) and crushed after adding a few drops of 2% potassium hydroxide. A drop of smear is taken on the glass slide and examined under a microscope with magnification of 600× (objective of 40× and eye-piece of 15×). Generally, 4-5 fields are observed in each smear. The pebrine spores will appear as oval shining bodies distinct from the other cells (Fig. 13).

A moth crushing machine which can crush 10-20 individual moths at a time, is also available. This facilitates proper and speedy crushing for quick examination (Fig. 14).

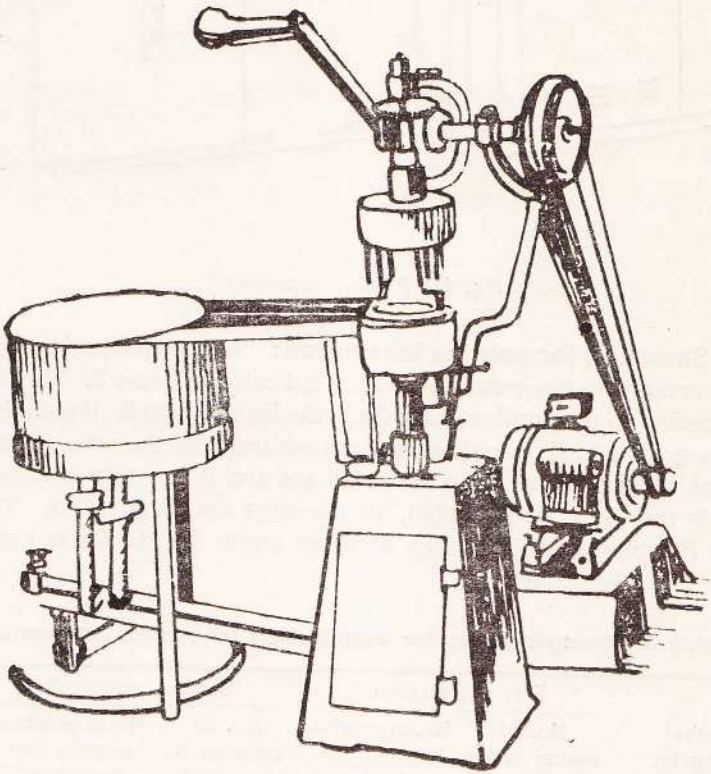


Fig. 14. Moth crushing machine

3.2. Mass examination: This method is suitable for quick and effective inspection of industrial seed. Mother moths are ground with cutter type mixer and spores are collected by centrifugation. Sample moths are collected at random from a batch. 20-30 moths are transferred to the crusher together with 90-100 ml. of 0.5% potassium carbonate solution and ground for 2 minutes at 10,000 r.p.m., to separate the spores from the tissue. After settling for 2 minutes, it is filtered and the filtrate is centrifuged at 2,600 r.p.m., (1,500 g) for 3 minutes for sedimentation of spores. The supernatant is rejected and the centrifugal sediment is dissolved in a few drops of 2% potassium hydroxide and taken for microscopic examination. Preferably, a phase contrast microscope is used for testing. Two smears from each sample are taken and five fields in each smear are examined. In a day of eight hours, about 500 samples

can be tested by this method. A sophisticated equipment is also available for this purpose (Hirano Pebrine Separator-Fig. 15).

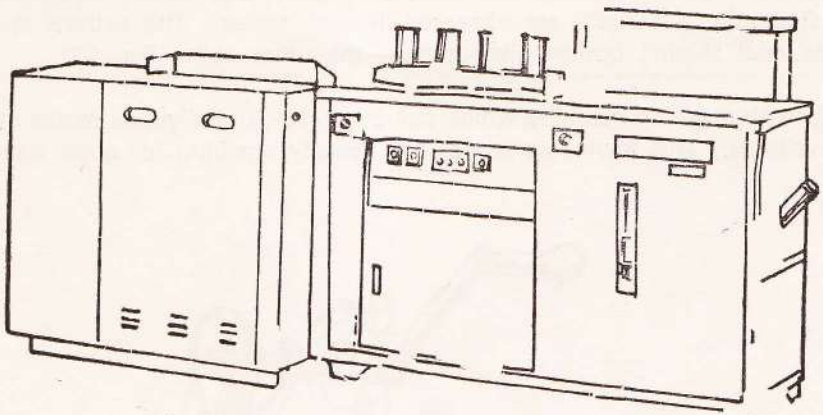


Fig. 15. Pebrine separator

3.3. Sampling for pebrine inspection: A standardised sampling method is followed for examining the industrial seed as indicated in Table 3. The moth samples are drawn according to the number of moths in the lot and tested. If there is no pebrine, all the eggs are qualified. If the pebrine smears are seen less than the number indicated in the table, the second examination is carried out and if the total number of infected smears exceeds the prescribed number, all the eggs are disqualified. Thus, if there are more than 700 moths in a lot, two samples are to be drawn as indicated in the Table 3.

Table 3. Sampling test for examination of pebrine in moths

No of moths in the rearing lot	First examination		Second examination		Total incidence for rejection
	No. of moths to be examined	No. of pebrine samples for rejection	No. of moths to be examined	No. of pebrine samples for rejection	
390 or below	All	1	—	—	1
391-500	390	1	—	—	1
501-600	450	1	—	—	1
601-700	480	1	1	—	1
701-800	565	2	105	2	2
801-1000	620	2	130	2	2
1001-2000	755	2	195	2	2
2001-3000	865	3	500	3	3
3001-4000	915	4	815	4	4
4001-6000	955	5	1140	5	5
6001-10000	990	6	1500	6	6
10001-30000	1030	6	1620	6	6
30001-or above	1060	6	1730	6	6

This method can also be adopted for examining industrial eggs prepared on cards. In the absence of the above equipment, the efficiency of testing could be increased by the use of grinder mixer combined with ordinary laboratory centrifuge.

4. EGG PROCESSING

4.1. Disinfection: Eggs on cards are soaked in 2% formaldehyde solution for five minutes, washed in water and dried to remove the contamination on the egg surface and to prevent dislodging of eggs during acid treatment. In case of egg cards, which are to be acid treated washing in water after formalin soaking is not necessary; the egg cards can be dried and taken for acid treatment. The use of high chlorinated bleaching powder during loose-egg preparation also serves as disinfectant.

4.2. Programming for hatching: Under natural conditions, bivoltine eggs do not hatch in 10-12 days after laying, since they enter into diapause. But they could be made to hatch from 10 days upto one year after laying, whenever desired. This can be achieved by treating the eggs with hydrochloric acid (hydrochlorisation) or storing them in low temperature (hibernation) or a combination of both.

4.3. Hydrochlorisation: There are various methods of treating the eggs with hydrochloric acid, using different strengths of acid, at different temperatures and durations. The commonly followed methods are as follows:—

4.3.1. Hot acid treatment: Hydrochloric acid of specific gravity 1.075 (15%) is heated to a temperature of 46.1°C and the silkworm eggs are immersed in the acid for 5-6 minutes. The specific gravity of the acid is as measured at 15°C. The acid strength varies with the temperature in which it is measured and therefore corrections are to be made as per Table 4.

For example, the acid of specific gravity 1.075 at 15°C will be 1.072 at 25°C and 1.064 at 46°C. The usual practice is not to heat the acid directly but to heat it in a hot water bath to maintain constant temperature (Fig. 16).

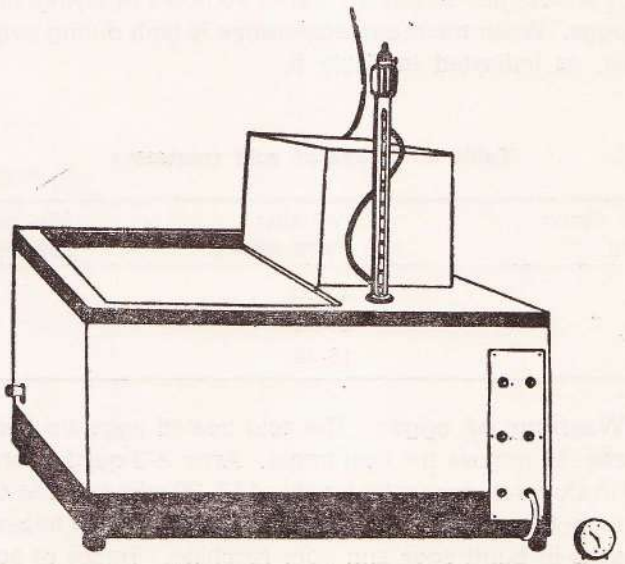


Fig. 16. Acid treatment bath

Table 4. Specific gravity correction of acid according to temperature

Specific gravity	°C	20	23	25	27	29	31	34	46	48
at 15°C (59°F)	°F	68	73	77	81	84	88	93	115	118
1.075		1.073	1.072	1.072	1.071	1.070	1.069	1.068	1.064	1.063
1.100		1.098	1.096	1.096	1.095	1.094	1.093	1.092	1.086	1.084
1.110		1.108	1.106	1.105	1.104	1.103	1.102	1.101	1.095	1.094

4.3.2. Cold acid treatment: The room temperature acid treatment is conducted at 25°C, using hydrochloric acid of specific gravity 1.10 (or 20%) for 60-90 minutes. If the room temperature is slightly higher, the duration of the treatment has to be reduced according to Table 5.

The room temperature treatment method takes longer time, but is safer. It is more economical as no heating is required. When egg cards are used, the eggs are, however, likely to drop off.

Table 5. Relation between room temperature and dipping duration

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

4.3.3. Time of treatment: The eggs are treated within 20-24 hours after laying. They should not be treated within 10 hours of laying, or after the change of colour of the eggs. When the room temperature is high during oviposition the eggs are treated earlier, as indicated in Table 6.

Table 6. Stage of acid treatment

Temperature during egg laying	Period after egg laying (hours)	Min. period after egg laying (hours)
24°C	20-30	10
27°C	20-25	10
29°C	15-20	10

4.3.4. Washing of eggs: The acid treated eggs are immediately washed in thoroughly water to remove the acid traces. After 2-3 quick changes of water, the eggs are washed in slow running water for about 15-20 minutes. The temperature of the water should be more than 20°C but less than 27°C. If the acid traces are not removed completely, it results in burnt eggs and poor hatching. Traces of acid, if any, on the eggs can be tested by using a blue litmus paper which turns red if not washed properly.

4.3.5. Egg drying: After washing, the eggs are immediately dried in shade. Drying is hastened under gentle air current. The temperature of the room should be maintained at 24-26°C. Keeping the eggs in wet condition for more than 10 hours is injurious. When handling large quantity of eggs, use of a hydro-extractor removes much of water and facilitates quick drying.

4.3.6. Postponement of acid treatment: When the acid treatment is desired to be postponed, the eggs can be stored at 5°C upto 5 days. In such cases, the eggs should be cold stored before 20 hours after egg laying. After taking out of cold storage, the eggs should be kept for an hour at room temperature before they are acid treated. Re-refrigeration of such layings after treatment should be avoided.

4.3.7. Postponement of hatching in acid treated eggs: Eggs treated as above incubated and hatch in about 10 days. The hatching could be delayed upto 20 days by cold storing (5°C) the treated eggs, 20 to 30 hours after treatment. The cold stored eggs can be released for incubation on any day within these 20 days. Refrigeration beyond 20 days results in poor hatching and should be avoided. Thus, by the above method, it is possible to make eggs hatch from 10 to 30 days after laying.

4.4. Acid treatment after chilling: The eggs can be made to hatch in 45 to 70 days after laying by cold storing and subsequent treatment with acid. The activation of eggs is initiated by cold storage and further hastened by acid treatment.

4.4.1. Hatching in 50-70 days: To obtain hatching in 50-70 days after egg laying, the eggs preserved at 25°C for 40-50 hours are cold stored at 5°C. The eggs can be released from cold storage any day between 40th and 60th day and acid treated. While cold storing or releasing, the eggs should be kept at 15°C for about 3 hours to avoid shock to the eggs. The eggs are taken out of cold storage and kept at 25°C for 3-4 hours and treated in acid of 1.10 specific gravity (at 15°C) at 47.8°C for 5-6 minutes, thoroughly washed in water and dried. The treatment should be done within 6 hours after release.

4.4.2. Hatching in 40-45 days: When hatching is required in 40-50 days after laying, the eggs are kept at 25°C for 30-35 hours and then cold stored at 5°C for 30-40 days. The method of cold storage and acid treatment are same as above. Longer the cold storage period, better will be the hatching. If eggs are taken out of cold storage before 30 days, the hatching is liable to continue for 3-4 days. Care should be taken to maintain the temperature at 25°C during and after oviposition. The age of the eggs before cold storage should be accurate.

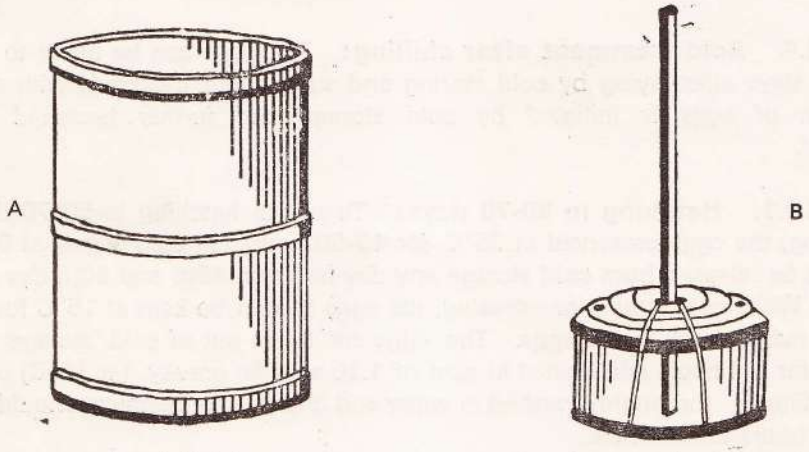
Hatching of eggs treated by the above methods can be delayed for about 8-10 days by refrigerating at 5°C within 12 hours after acid treatment. Before re-refrigeration, the eggs must be kept at 15°C for 6-12 hours. However, such re-refrigeration is not desirable.

4.5. Acid preparation: Commercial grade hydrochloric acid can be

used. Generally the specific gravity of the commercial acid is about 1.18. It should be diluted to the required concentration by adding water. A narrow range hydrometer (1.00 to 1.10 sp. gravity) is used for measuring the specific gravity. The temperature of the acid is measured and accordingly the required strength of acid is prepared as indicated in Table 4. The acid can be reused after filtering and correcting the specific gravity.

4.5.1. Practical aspects: It is preferable to have two vats of acid so that the eggs are first dipped in one vat and immediately transferred to the other. This prevents the decrease in concentration of the acid during treatment. When the temperature of the acid is higher than required, a polythene bag containing ice cubes can be dipped in the acid to lower the temperature.

The egg cards should be treated in convenient numbers, held together by a string or placed in special plastic frames, to facilitate the flow of acid between the sheets. The acid must be stirred frequently during the treatment to maintain uniform temperature. Loose eggs are tied in porous cloth bags or placed in perforated plastic containers (Fig. 17) The pores should permit the entry of the acid but should be smaller than the size of the eggs.



A. Acid tub

B. Perforated plastic container

Fig. 17.

4.6. Preparation of loose eggs: Silkworm eggs have a gluey coating and adhere to the egg card when laid. If the eggs are laid on starched paper, they detach easily when soaked in water. Lawn cloth or craft paper sheets are coated with a thin layer of edible starch (arrowroot) and dried. The moths are allowed to lay eggs on these starched sheets. 500-600 moths are allowed per square metre, within a wooden frame, about 3 cm high to restrict the moths.

The egg sheets are soaked in water for about 30 minutes and the eggs are gently brushed under a stream of running water on an inclined board and are collected in a perforated container. The eggs are soaked in 0.5% bleaching powder (calcium oxychloride) solution for 10 minutes to remove the glue from the eggs and to disinfect them. After thorough washing in water the eggs are acid treated, if required. To

separate unfertilised eggs, the eggs are transferred into a transparent, conical, plastic container shaped like a separation funnel, filled with salt water solution of 1.10 specific gravity. The unfertilised eggs sink to the bottom, which are drawn out by opening the tap. The process is repeated number of times, with successive lowering of the specific gravity till all the unfertilised eggs are removed.

The eggs are again thoroughly washed in water, drained and spread over a cotton cloth in a thin layer to dry. The eggs are sieved to remove the clumps and make them free-flowing. A sample is weighed to determine the number of eggs per gram and to determine the weight of 20,000 eggs. Eggs are weighed and poured into the egg case through a funnel and the case is sealed. The case is generally made of thin muslin cloth, pasted on to a wooden frame (Fig. 18) and usually holds 20,000 eggs.

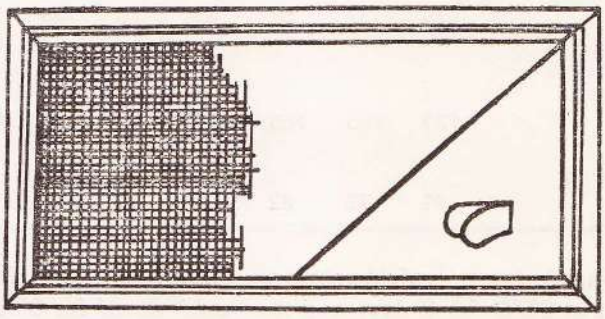


Fig. 18. Loose egg container

Loose silkworm eggs facilitate easy handling, saves storage space during incubation and cold storage, and fixing of units for distribution. On the other hand, preparation of eggs on cards is simpler but the number of silkworm eggs supplied cannot be accurately assessed. In many countries, the industrial eggs are sold in loose form.

4.7. Hibernation of bivoltine eggs: In univoltine and bivoltine races, the eggs enter into diapause in 40-50 hours after oviposition at 25°C. Eggs when laid are yellow in colour. Gradually, they change to light brown and then to purplish brown. However, this change of colour depends on races and seasons. Such eggs do not hatch unless they are activated by cold temperature. This is naturally achieved in temperate areas because of marked winter conditions. In tropical areas, the winter conditions could be simulated by preserving the eggs at required temperatures in a cold storage. Such cold storage of eggs is carried out by following specific schedules. Low temperature preservation terminates the diapause and activates eggs to hatch. Cold storage can also be used to check further development in activated eggs.

The schedule comprises three distinct phases viz., aestivation or storing at 25°C, cold storage at 5°C and 2.5°C and an intermediate phase, where the temperature is gradually lowered from 25°C to 5°C. Humidity plays a vital role during

these stages. A relative humidity of 75-80% is ideal. Aestivation at higher temperature of 30°C and 95% humidity results in high mortality (even upto 60%) in the early and late larval stages.

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 7.

Table 7. Relationship between days preserved at high temperature (25°C) and low temperature (5°C)

No. of days preserved at high temperature.	1	3	5	10	20	40	60	80	100
Minimum period of cold storage required for 90% hatching (days).	79	87	87	89	98	105	119	124	134
Maximum period of cold storage to obtain 90% hatching (days).	139	165	168	178	193	202	196	161	145
Effective period of cold storage (days).	61	79	82	90	96	98	78	38	12

4.7.1. Short term refrigeration (Refrigeration for 3 months): The eggs are kept at 25°C for 3 days and then cold stored for 90 days at 5°C. The cold storage and release of eggs should be through an intermediate temperature of 15°C for 6-12 hours.

4.7.2. Refrigeration for 4 and 6 months: The preservation period at different temperatures is given below:—

Table 8. Hibernation schedule for 4 & 6 months

(Days)

	Temp °C						
Hibernation Period ↓	25	20	15	10	5	2.5	15
4 months	10	2	2	3	50	50	1
6 months	20	15	10	10	50	60	1

The eggs stored according to the above table can be released 15 days earlier or later than the scheduled period, without affecting the hatching. After releasing the eggs from 2.5°C, the eggs must be kept at an intermediate temperature of 15°C for one day and then transferred to incubation room.

4.7.3. Long term refrigeration: The schedule for preserving the eggs for 10 months is given in Table 9.

Table 9. Schedule for preserving bivoltine eggs for 10 months

(Days)

Hibernation Period ↓	Temp °C							
	25	20	15	10	5	2.5	15	2.5
10 months	50	40	25	25	60	55	4-5	30-40

Each embryonic stage has different sensitivity to low temperature and cannot withstand a long continued refrigeration at one stretch. As such, a two step refrigeration has to be carried out to preserve the eggs for long periods. After the termination of diapause, the eggs are allowed to develop upto the 'longest embryo' stage (Fig. 19), which can tolerate further cold storage. Thus, intermediate care of exposing the eggs to 15°C for 4-5 days is followed to attain the longest embryonic stage, after which the eggs are again refrigerated at 2.5°C. Intermediate care is given for 40-50 days, before the expected day of hatching.

5. INCUBATION

For healthy development and uniform hatching, bivoltine eggs are to be incubated under optimum conditions of temperature, humidity and light. Hibernated eggs are released for incubation through intermediate temperature (15°C). After release they have to be soaked in 2% formalin for 5 minutes, washed in water and dried before incubation. The egg cards should be spread in single layers in the trays. In case of loose eggs, they must be thinly spread out in brushing frames. Incubation room and the equipments used should be thoroughly cleaned, washed and disinfected before the start of incubation.

Incubation has a profound influence on the voltinism, larval health, as also on the yield and quality of cocoon crop. To obtain maximum hatching, optimum incubation temperature is 25°C which is raised by 1°C to 26°C, on the day of hatching. During incubation, humidity is maintained at 75-80% as excessive dryness results in dead eggs, poor hatching and weak larvae. Higher humidity causes weak larvae, although it makes hatching uniform. As the embryo grows most vigorously during incubation, good ventilation should be provided. Care has to be taken not to overcrowd the eggs in a narrow incubation room. Light should be provided for 18 hours a day, till head-pigmentation stage is reached.

Eggs incubated as above hatch in 10-12 days. Two days before hatching, the colour of the egg changes to a lighter shade, with a distinct dark spot. This is the 'head pigmentation stage'. One day before hatching, the eggs turn bluish, which is referred to as 'body pigmentation' or 'blue egg stage'. These two stages are very sensitive to low humidity.

To obtain uniform hatching, the eggs are kept in darkness at the head pigmentation stage. Darkness arrests hatching of the developed eggs and facilitates lagging embryos to reach the hatching stage. After two days, when a few larvae hatch out, the eggs are exposed to light. This ensures uniform hatching.

5.1. Refrigeration of 'blue eggs' and 'new born larvae': Hatching of eggs under incubation can be delayed if required by refrigerating them in the 'blue egg' stage at 5°C for 2-3 days. Care must be taken to provide 75-80% humidity during cold storage. New born worms can also be refrigerated at 5°C for 2-3 days but this is not desirable.

6. PACKING & TRANSPORTATION OF EGGS

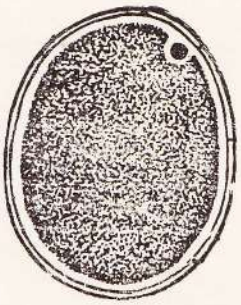
For convenience in handling, the eggs are packed properly so as to avoid any damage or injury to the developing embryo. Head pigmentation and blue egg stages are very sensitive especially to low humidity; therefore transportation of eggs during these stages should be avoided. It is safe to transport them before 5th day of incubation. The eggs are transported preferably in cooler hours to prevent desiccation during hotter periods of the day. Boxes made of wood or thermocole with adequate ventilation are preferred. Placing a wet strip of foam pad inside the box helps in increasing humidity but it should not wet the eggs. The eggs should be loosely packed in convenient sized boxes.

7. EMBRYO TESTING

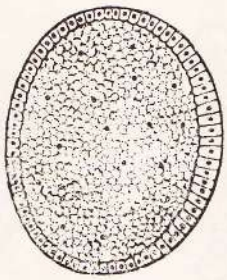
The various processing and preservation methods of eggs are followed essentially at the specific stage of embryonic development. The following is a simple technique useful to identify the various stages of embryonic development:—

A sample of about 30 eggs are immersed in a boiling solution of 2-3% potassium hydroxide for 10-15 seconds. They are then transferred to hot water (60°C) and the water is squirted strongly on the eggs. The embryos get separated from the egg shell and float, which can be removed by using the squirt. The embryos are transferred to 50% alcohol, stained with borax carmine and examined under the dissection microscope. The eggs or the embryos should not be left for longer periods in potassium hydroxide or hot water, as they disintegrate and dissolve.

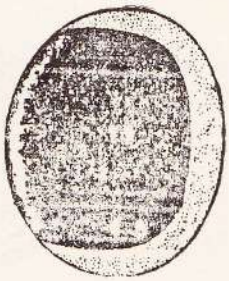
The different developmental stages of the embryo are explained diagrammatically (Fig. 19).



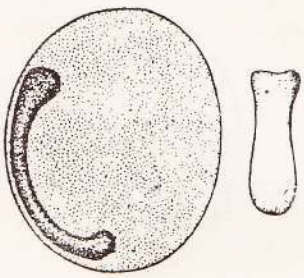
Structure of egg



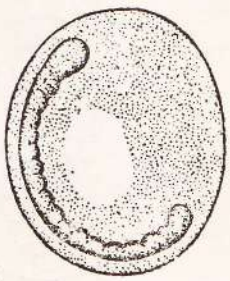
Blastoderm formation



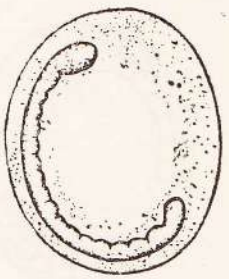
Formation of germ band (right time for common Acid treatment)



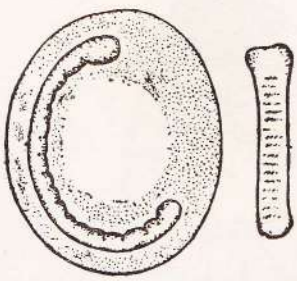
Spoon shaped embryo (right stage for "chilling")



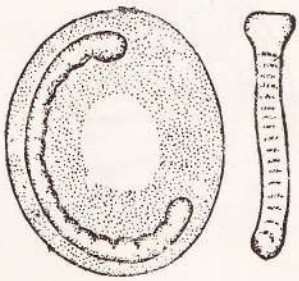
I
Diapause stage



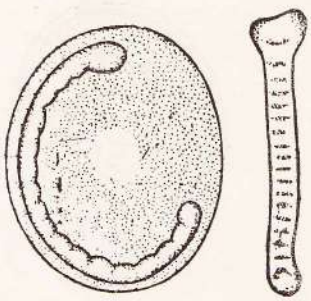
II
Diapause stage



Ko-stage



Otsu-A

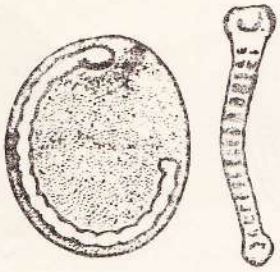


Otsu-B

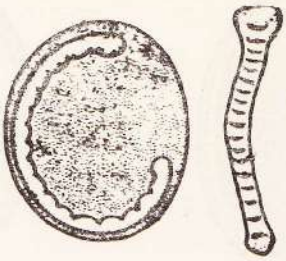
(STAGES DURING COLD STORAGE)

Fig. 19. Different stages of embryonic development

Contd.

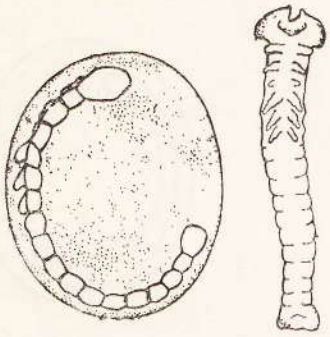


Hel-A

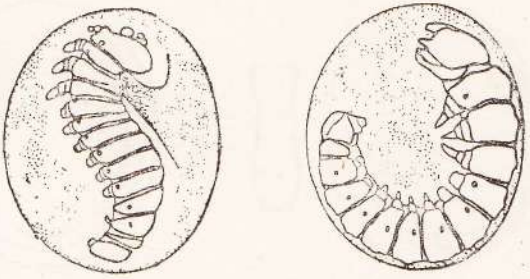


Hel-B

LONGEST EMBRYO STAGES
(STAGES ATTAINED DURING INTERMEDIATE CARE)
(DOUBLE REFRIGERATION)

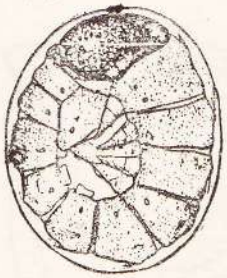


Appearence of thoracic legs



Blastokinesis-stages (Embryonic inversion)

Head pigmentation stage



Blue egg stage

Hatching

Fig. 19. Different stages of embryonic development

Table 10. Schedule for hatching at different periods

Days after laying when hatching is required*	Method to be employed
10 to 30 days	Acid treatment and cold storage at 5°C upto 20 days.
40 to 50 days	30-35 hr at 25°C, 30-40 days at 5°C and acid treatment.
50 to 70 days	40-50 hr at 25°C, 40-60 days at 5°C and acid treatment.
70 to 85 days	Same as above and then cold storing of eggs at 5°C after treatment.
100 to 120 days	3 days at 25°C and 90-110 days at 5°C.
120 to 140 days	10 days at 25°C, 3 days at 20°C, 3 days at 15°C and 10°C, 50 days each at 5°C and 2.5°C.
175 to 205 days	20 days at 25°C, 15 days at 20°C, 10 days each at 15°C and 10°C, 50 days at 5°C and 60 days at 2.5°C.
295 to 315 days	50 days at 25°C, 40 days at 20°C, 25 days each at 15°C & 10°C, 60 days at 5°C, 55 days at 2.5°C. 4-5 days at 15°C and 2.5°C for 30-40 days

*Incubation period included

IV. MANAGEMENT OF INDUSTRIAL BIVOLTINE GRAINAGE

Grainage management aims at production of high quality dependable seed efficiently and economically. This involves technical as well as managerial skill. Grainage management comprises mostly of market survey, production planning, quality control, economising the production cost and follow-up. The grainage has to build a reputation by virtue of its technical excellence in producing quality seed. It should earn the confidence of sericultural farmers. Like agricultural seed, silkworm seed also sells on the reputation of the grainage, which must be developed and guarded zealously.

1. LOCATION OF THE GRAINAGE

Usually, industrial grainages are located in the cooler areas of the sericultural belt. The advantage of such location lies in reducing the cost of cooling which is more than the cost of heating a room for increasing the temperature as the optimum temperature of grainage activity is about 25°C. But in tropical areas, this limitation has to be accepted by equipping the grainage suitably.

It will be ideal, if the grainages are located in the heart of the sericulturally concentrated areas to enable quick transportation of eggs or larvae. If the grainage is located at a far-off place, it might be necessary to have a number of incubation centres or sales points to supply the seed to rearers. This arrangement may prove expensive. It is cheaper and convenient to locate it near the sericultural areas and to equip it suitably. The industrial grainage cannot be located in seed areas which are isolated by seed legislation.

The grainage should be located where basic facilities like water, power, labour, transport and adequate space for construction of the building are available.

2. MARKET SURVEY

The grainage manager should study the rearing magnitude during different seasons, requirement of seed for each season, the choice of the farmers for the silkworm races as also the performance of the seed crops. It would be desirable to know the above so as to be able to forecast the need. This forms the starting point for planning.

3. PRODUCTION PLANNING

3.1. Programme of production: The production of 25 lakh dfls envisaged per year is not distributed evenly over 12 months. It depends on the demand for eggs, seasons favourable for seed crop rearing and egg production. The planning should be so adjusted that major quantity of eggs are produced during favourable seasons and programmed for availability as per demand.

Production should be planned well in advance, as the minimum time required to produce a batch of seed is two months. The rearing and production of seed cocoons should suit the programme of egg production. It will be convenient to have three batches of production per month. The rearing of parental races is required to be planned accordingly.

A forecast of egg production is possible at the time of seed cocoon preservation as also during emergence period. One kilogram of seed cocoons (about 600 cocoons) can yield approximately 4 cases of eggs or 200 dfls. The number of moths left for egg laying will also indicate the expected production. The dfls produced will be about 90% of the number of pairs.

3.2. Arrangement of parent seed: The supply of parent seed to the seed rearers must be arranged by the grainage. The parental seed for the production of seed cocoons is generally called P1 seed and is obtained from a government agency or the breeding organisation. In India, P1 seed is produced only by government agencies and not by the industrial/commercial grainage, which will ensure the production of disease free quality seed. The industrial grainage should procure and incubate the P1 seed of the required races and distribute to its seed rearers.

The grainage should adopt the seed rearers for getting quality seed cocoon. It is essential that the seed rearers are located away from the industrial rearing areas preferably where climate is favourable. Adequate irrigation facility should also be available for mulberry cultivation.

The number of seed rearers enrolled by the grainages depends on the quantity of industrial seed to be produced. For production of every 1,000 dfls (or about 20 cases) of hybrid seed, 20 dfls of parent seed are to be reared.

3.3. Selection of seed rearers: An ideal seed rearer should have limited rearings for obtaining quality seed cocoons. A rearing of about 100-150 layings per batch is considered ideal. He should be highly experienced, skillful and conversant with modern technology of rearing. He should have the basic facilities of good mulberry garden, rearing accommodation and equipments and must be conscious about the importance of seed cocoons he produces. These seed cocoon rearers are better located in small groups in 2 or 3 villages nearby to have technical guidance from the grainages. Where distinct seed areas are organised under legislation, it will be necessary that the selected seed rearers are located within their boundaries. The selected seed rearer must be discouraged to rear the industrial seed. To ensure this, the seed rearers have to be provided with adequate incentives in the form of rearing equipments, inputs, assured price and bonus for the seed cocoon and prompt payment etc.

3.4. Extension support: It will be in the interest of the grainage that the seed cocoon rearer is guided suitably and extension support is provided. This will help in producing a good seed crop which in turn assures higher egg yield. The seed crop is to be periodically monitored for detection of diseases. Late moulting and undersized larvae in different stages should be microscopically examined. If pebrine is detected, the crop should not be accepted for seed preparation. Thorough and effective disinfection should be assured before the next crop.

3.5. Incentives: To induce quality consciousness amongst the seed rearers, norms have to be fixed regarding effective rearing ratio, percentage of healthy pupae, number of cocoons per kilogram, etc. The norms have to be fixed considering the season; for example, in favourable seasons, the norms for a seed crop can be: cocoon weight of 1.5 g and above; percentage of live pupae 90% (average of 200 cocoons), etc., Those who produce better cocoons should be given an incentive bonus. By a system of bonus and incentives, the seed rearers can be induced to produce high quality seed cocoons which very much simplifies the task of industrial grainage in the production of quality hybrid seed.

4. PRODUCT MIX

More than one hybrid combination may have to be produced to suit the region or the season. For the same season in an area, more than one hybrid may be required depending on the preference of the farmers. Accordingly, the grainage management should plan its product mix so that there should not be shortage of one and an unsold excess of the other hybrid. This has to be planned carefully, based on the performance of the hybrids, consumer pattern and market survey.

Although both regular and reciprocal crosses are designated and marketed under one name, preparation and distribution of these two hybrids should be separate for sake of uniformity in larval development and cocoon character.

5. DISTRIBUTION OF SEED

5.1. Distribution of incubated eggs: Studies have shown that more than 60% cases of hatching defect are due to improper incubation. The eggs produced with all care, if incubated in a faulty manner by the farmer, may not hatch satisfactorily, which will ultimately affect the reputation of the grainage. To protect the interest of the grainage and to ensure good hatching, it will be a better management practice to incubate the eggs and distribute hatched larvae to the farmers. This, no doubt, involves extra effort and cost, but it is essential. The grainage could even charge an extra fee, for incubation.

A chain of incubation centres may be necessary as transportation of hatched larvae over long distances is not desirable. These centres besides serving as sales points will also help in maintaining liaison with the farmers for follow-up and feed back. Maximum care during egg handling is necessary as newly hatched larvae are highly susceptible to infection, especially to the fungal diseases.

5.2. Distribution of chawki reared worms: Success of rearing depends to a great extent on the care bestowed during young age. Since many of the farmers lack the facilities and technology required for proper rearing of young worms, well equipped Community Chawki Rearing Centres are most essential. The grainage could take advantage of these centres and co-ordinate with them. Incubation facilities could be organised in the Chawki Rearing Centres. It would also facilitate distribution and avoid transportation of new-born larvae over long distances. This will ensure satisfactory hatching, proper chawki rearing and successful cocoon crops.

6. QUALITY CONTROL

The success of the grainage depends on the quality of the eggs produced, its uniform hatching and subsequent success of cocoon crop. These could be realised only through strict quality control at all levels of operation. There are four important stages during which the quality of eggs is likely to be affected and therefore, require maximum care and attention. These are: production, preservation of seed cocoon, egg processing and incubation.

6.1. Seed cocoon production: The quality of the seed cocoon has profound influence on the industrial seed produced. Special attention must be paid to the following aspects:

6.1.1. Rearing of seed crop should be well planned to produce synchronising batches of cocoons and to minimise refrigeration of pupae and moths.

6.1.2. The parent seed must be totally pebrine free and supplied at the required time.

6.1.3. The parent seed should be incubated properly, preferably chawki reared and distributed under ideal conditions.

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6.1.4. Disinfection of the rearing room and equipments should be thorough and effective.

6.1.5. Due attention should be paid for proper rearing and mounting of seed crop. The cocoons should be harvested only after complete pupation.

6.1.6. The seed crop should be screened for pebrine by examining the larvae at all stages.

6.1.7. The seed cocoons should conform to the norms of pupation rate, yield and cocoon characters.

6.1.8. The races should not be mixed and preferably one race is to be reared by one rearer.

6.1.9. Seed cocoons should be transported preferably by the grainage itself to ensure all safety measures.

6.2. Seed cocoon preservation

6.2.1. Seed cocoons received from different farmers should be preserved and processed separately.

6.2.2. The defective cocoons should be sorted out and pupae tested for pebrine.

6.2.3. Forced eclosion test should be conducted for early detection of pebrine.

6.2.4. Sexing of pupae is essential to produce true hybrid seed.

6.2.5. Optimum temperature and humidity are to be maintained during the preservation of pupae/cocoons.

6.2.6. The emergence of component races should be synchronised. Refrigeration of female pupae or moth should be avoided.

6.2.7. A minimum of 4 hours of copulation should be ensured.

6.2.8. Male moths are to be cold stored at proper temperature and should not be used more than twice.

6.2.9. Proper conditions should be ensured for uniform emergence of moths.

6.2.10. Optimum conditions of temperature, humidity and light during coupling and oviposition should be provided.

6.3. Egg processing

6.3.1. Examination of mother moths should be conducted scrupulously and the seed which is not in conformity to the set norms of moth testing should be destroyed.

6.3.2. The eggs should be treated within 20-24 hours of egg laying and before the change of colour.

6.3.3. During acid treatment, the specific gravity and the temperature of the acid and duration of treatment should be strictly followed.

6.3.4. The hydrochlorised eggs should be washed in water thoroughly.

6.3.5. The hydrochlorised eggs, if necessary should be refrigerated at the right time and temperature and for not more than 20 days.

6.3.6. The duration of hibernation should be planned in advance and suitable schedule should be followed strictly.

6.3.7. While cold storing or releasing from cold storage, the eggs should pass through an intermediate temperature of 15°C.

6.3.8. The embryonic development of eggs must be monitored regularly through embryo tests. Cold storing and 'intermediate care' should be followed at appropriate stages of development.

6.3.9. Samples should be drawn from each lot of eggs produced for assessing the quantity of dead eggs, unfertilised eggs, etc. and also to test hatching performance.

6.3.10. Hygienic conditions should be rigidly observed at all stages of seed production.

6.4. Incubation

6.4.1. Hygienic conditions during incubation should be ideal and all the equipments, room, etc., should be effectively and thoroughly disinfected.

6.4.2. The eggs should be disinfected before incubation.

6.4.3. Proper temperature, humidity and light conditions during incubation should be ensured.

6.4.4. Overcrowding of eggs or egg cards should be avoided.

6.4.5. The eggs should be transported within the first 4-5 days of development and should not be transported in head-pigmentation or blue egg stage.

7. SAFETY MEASURES

The efficiency of egg production lies with the workers engaged in seed production. It is the responsibility of the grainage management to protect the health of the workers from the hazards involved during the process of egg production. Moth scales, formalin and acid fumes cause health hazards to the workers.

The moth scales or moth dust liberated during emergence, coupling and decoupling irritate mucus membrane of the nose and create discomfort. To protect against this, the workers should be provided with face masks to cover the nose and mouth. The fumes of formalin used during disinfection and egg processing are sharp irritants to the nose, eyes and skin. Suitable masks and hand-gloves are to be provided to workers while handling formalin. Acid treatment equipment must be kept in a well ventilated space and workers must be provided with protective wear. The acid fumes, corrode the electrical equipment, metal etc., and hence should be isolated from other parts of the grainage. Adequate exhaust fans must be provided in the grainage.

8. ECONOMISING THE COST OF PRODUCTION

The cost of production of eggs in an industrial grainage can be brought down by increasing the egg yield, adopting labour saving practices and reducing losses due to unsold seed or rejection of eggs due to diseases. The factors contributing to high egg yield are high pupation rate, good cocoon weight and races with high fecundity. Increase in the percentage of emergence of moths, percentage of laying and reduction in the unfertilised or dead eggs are controlled by the environmental conditions of the grainage. These contribute to increased egg yield and consequently reduce the cost of production.

The unit of egg production should be well planned for effective utilisation of manpower. Use of labour saving equipments for cocoon cutting, sexing, moth examination etc., reduces cost of production. Preparation of loose eggs saves labour and space in refrigeration and incubation. Proper seed crop rearing will minimise rejection of eggs due to pebrine. By adopting the forced eclosion method, pebrine disease, if any, can be detected well in advance. This saves the cost of seed cocoon, labour and material. In areas where multivoltine races are also reared, the bivoltine male moths can be reused for preparing hybrids with multivoltine. This will reduce the cost of production significantly. The cocoon shells should be stored separately under proper conditions and protected against dermestid beetles to fetch better price.

9. RECORDS

It is essential that accurate data of seed production are maintained in the grainage. This will help in efficient management, control of quality and in solving practical problems. The following are the types of records that will be useful and are to be maintained. In all the records, the details of temperature and humidity are to be noted.

9.1. Seed cocoon record: The register should contain information on the name of the rearer, source of parent seed, race, performance of the crop, result of pupal testing, date of spinning, quantity, cocoon assessment data, percentage of rejection, pupation rate and the quantity of cocoon kept for seed production. This record determines the price and the premium to be paid to seed rearers.

9.2. Emergence record: Details on the emergence, such as number of moths obtained each day, number of pairs, percentage of emergence, number of moths left for egg laying under each combination etc., are recorded. This will help in forecasting the production and planning for hibernation or acid treatment. The data are maintained separately for different batches.

9.3. Moth examination record: Maintenance of this record is important. It provides information on the number of moths examined, type of examination, result, incidence of pebrine, if any, percentage of infection, whether the batch is to be rejected etc. The batches fit as seed are to be certified.

9.4. Egg production record: Information pertaining to the quantity of eggs laid, unfertilised eggs rejected, quantity fit for distribution, are recorded. The record should also indicate the quantity of eggs acid treated, hibernated or refrigerated.

9.5. Hibernation and refrigeration record: Details of refrigeration, aestivation, hibernation, date of release, temperature in cold storage etc., are required to be precisely recorded.

Records on collection of indents, incubation, distribution of seed and crop performance etc. are also necessary.

10. COST STRUCTURE

The cost of establishing and operating an industrial bivoltine grainage to produce 25 lakh dfls per year is discussed. The capital investment on land, building, cold storage, etc. comes to Rs. 25.80 lakh, while non-recurring expenditure on equipments, furniture and vehicle Rs. 4.56 lakh. Expenditure on salaries & wages, consumables, electricity and other operational cost works out to Rs. 5.08 lakh. These details are given in Annex 1. Cost of grainage equipments is given in Annex 2. The staff pattern is presented in Annex 3.

10.1. Cost of production: The cost of seed production includes the interest on capital investment, depreciation on buildings & equipment and expenditure on seed cocoons, consumables, salaries of staff, workers' wages etc. The receipts of the grainage are the sale proceeds of the eggs and rejected and cut cocoons.

Because of high variability in the cost of inputs in different regions and countries, the cost of seed has not been worked out. However, the preceding details can serve as a guideline.

Annex 1: Cost structure of the grainage

Particulars	Amount (Rs)	
Capital investment		
1. Land (1 ha @ Rs. 30,000/-ha)	30,000	
2. Grainage building	17,00,000	
3. Cold storage	6,00,000	
4. Office building	2,00,000	
5. Bore well	50,000	25,80,000
Non-recurring expenditure		
1. Grainage equipment	3,05,600	
2. Furniture & fixtures	50,000	
3. Vehicle	1,00,000	4,55,600
Recurring expenditure		
1. Establishment charges	2,86,200	
2. Cost of chemicals, (HCl., Formalin, KOH etc.)	10,000	
3. Cost of egg sheets/egg cases	25,000	
4. Worker' wages, [20 workers @ Rs. 10/- per day (20×30×12×10)]	72,000	
5. Stationery & printing	10,000	
6. Electricity, diesel & water charges	60,000	
7. Maintenance of vehicle	10,000	
8. Contingencies	10,000	
9. Miscellaneous	25,000	5,08,200
Grand total		35,43,800

Annex 2: Cost of grainage equipments

Sl. No.	Particulars	Amount (Rs)
1	Cocoon preservation stands (Wooden) 60 nos. @ Rs. 220/- each.	13,200
2	Bamboo round trays 4' diameter 600 nos. @ Rs. 15/- each	9,000
3	Grainage trays (plywood bottom) (3'×2'×3½") 400 nos. @ Rs. 60/- each	24,000
4	Working stands 16 nos. @ Rs. 40/- each	640
5	Moth examination tables (6'×2½×2½') 5 nos. @ Rs. 400/- each	2,000
6	Stools (Wooden 18"×18"×2') 20 nos. @ Rs. 75/- each	1,500
7	Ant wells, 300 nos. @ Rs. 5/- each	1,500
8	Cellules 1,50,000 nos. @ Rs. 85/1,000 nos.	12,750
9	Hirano pebrine separator No. 1	1,37,500
10	Hygrometers 20 nos. @ Rs. 100/- each	2,000
11	Hydrometers 4 nos. @ Rs. 30/- each	120
12	Thermometers 4 nos. @ Rs. 50/- each	200
13	Acid treatment baths 4 nos. @ Rs. 4,500/- each	18,000
14	Slide carriers (wooden) 150 nos. @ Rs. 5/- each	750
15	Basin stands 12 nos. @ Rs. 50/- each	600
16	Sprayers 3 nos. @ Rs. 600/- each	1,800
17	Cocoon cutting machine 1 no. @ Rs. 50,000/- each	50,000
18	Cocoon deflossing machines 2 nos. @ Rs. 10,000/- each	20,000
19	Microscopes 10 nos. @ Rs. 1,000/- each	10,000
	Total ..	3,05,560

Annex 3: Staff pattern

Sl. No.	Designation	Scale (Rs)	No. of posts	Salary/ month/ Staff (Rs)	Total/ year (Rs)
1	Grainage Officer	(700—1300)	1	1,820	21,840
2	Technical Assistants	(550—900)	3	1,360	48,960
3	Assistants	(380—560)	12	890	1,28,160
4	Clerks	(330—560)	2	850	20,400
5	Driver	(260—350)	1	640	7,680
6	Watchmen	(190—232)	3	450	16,200
7	Mechanic	(425—700)	1	1,100	13,200
8	Asst. Mechanic	(260—430)	1	700	8,400
9	Seed-crop Supervisor	(380—560)	2	890	21,360
		Total ..	26		2,86,200