PATHOLOGY OF NON MULBERRY SILKWORMS

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The scientific study of the nature of disease and its causes, processes, development, and consequences. Also called *pathobiology*.

1. PATHOLOGY OF TASAR SILKWORM

Nearly 35 to 40% of the tasar crop is lost from disease. The tasar silkworm is subject mainly to four types of diseases.

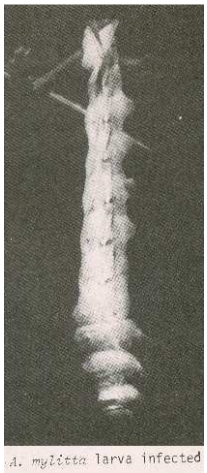
They are 1. polyhedrosis (virosis) 2. Bacteriosis

3. Mycosis and 4. Microsporidiosis.

1. Polyhedrosis (virosis)

Symptoms: The infected larva becomes soft and sluggish. In about 18 hours the body loses its natural shape, becoming distended and swollen. The integument turns opaque and brownish, and the internal tissues disintegrate. The larva dies about 24 hours after the appearance of the symptoms.

After death it hangs head down, remaining attached to the plant twig only by its claspers (Figure), and dark brown fluid oozes in drops from the mouth. The skin becomes fragile and blackish. The dead larva emits an obnoxious odour and becomes a mass of melted tissues.



A. mylitta larva infected with polyhedrosis

Infected individuals may succumb during spinning, after pupation or even at emergence. The dead pupae "melt", generally dirtying the cocoon. The life span of infected adults is shortened and their fecundity reduced.

Causative agent: This virus disease is characterized by the presence of polyhedral inclusion bodies, which are cytoplasmic (CPV). They are highly refractile crystalline bodies occurring singly or in groups and ranging from 3 to 8 microns in size. The crystals are usually hexahedral, although various shapes, from trideral to octahedral, may also be seen.

An electron micrograph of a polyhedron reveals a socket •. also six-sided, in which the virus particles reside. The double shadowing technique has shown that the cytoplasmic virus of *A. mylitta* is icosahedral.

Transmission: The most common mode of transmission is oral; but transovum transmission through surface contamination of the eggs laid by infected females has also been observed. The larvae contract the disease when they eat a portion of the eggshell during hatching.

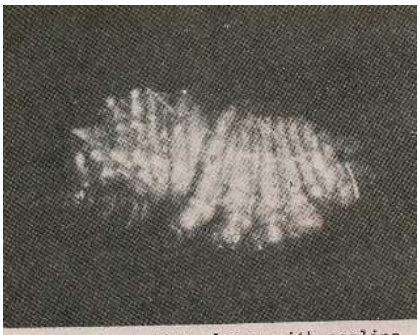
Incidence: The estimated loss in rearings due to the disease is 25%. The loss is higher in the first rearing season than in the second, and in the third it is minimal. The incidence of mortality increases with age.

Predisposing factors: High humidity and temperature cause higher mortality from polyhedrosis. The natural epizootics of the disease were found to be more common in higher density populations. Feeding on tender, juicy leaves which are deficient in nitrogen increases susceptibility. Besides, an imbalance between nitrogen and carbohydrates in the food induces polyhedrosis.

2. **Bacteriosis**

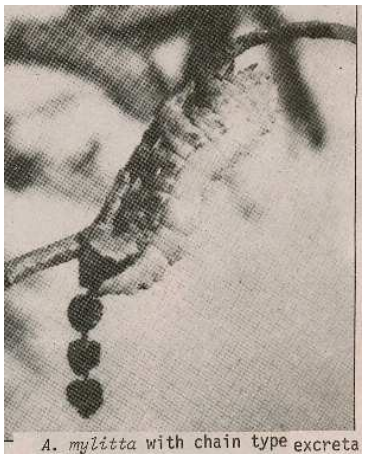
The tasar silkworm suffers from three types of bacterial disease with three distinct Symptoms.

1. **Sealing of the anal lips (SAL):** The larva first becomes restless. In about 12 hoursit loses appetite and becomes sluggish. In another 12 hours it starts defecating a soil-coloured, sticky, semisolid excreta which solidifies on contact with air, sealing the anal lips completely (Figure). At this stage the larva stops feeding, becomes immobile and shrinks lengthwise. In another 8 to 10 hours it loses its grip on the twigand topples. The larva dies in another 24 hours.



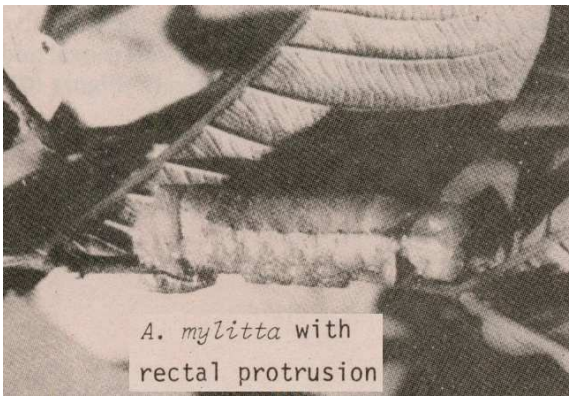
A. mylitta larva with sealing of the anal lips

2. **Chain type excreta (CTE):** The diseased larva is slightly thin, soft and sluggish and engages in limited feeding activity. In about 24 hours it starts to excrete a slimy substance along with the faeces, as a result of which chain of faecal beads hangs from the anal opening (Figure). The claspers lose hold of the twig, and the posterior half of the larva hangs obliquely. It remains in this position for about 12 hours before toppling and dying.



A. mylitta with chain type excreta

3. **Rectal protrusion (RP):** The infected larva becomes restless and sluggish. In 18 to 20 hours the rectum is protruded in the form of a transparent bag filled with green haemolymph (Figure). The anal lips dilate, feeding stops and the body contracts lengthwise. The larva remains attached to the twig for 6 to 8 hours before falling. Itdies in another 24 hours.



Causative agent: Microscopic examination of the infected larvae reveals the presence of gram-negative micrococci (in singles and chains) and gram-positive bacilli (thin isolated rods and large rods in chain formation).

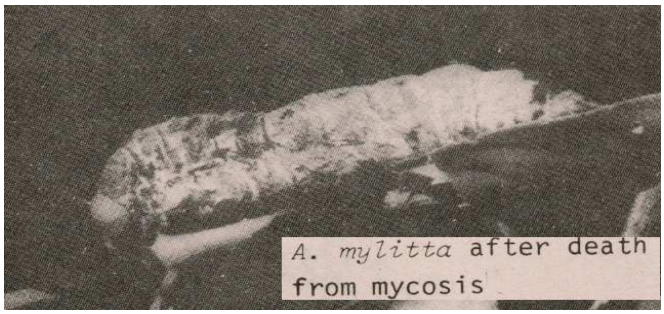
Incidence: Bacteriosis causes about 15% of larval mortality. Among the bacterial diseases, the incidence of SAL is highest (8%), followed by CTE (5%) and RP (2%). The incidence of bacteriosis is higher in the second rearing season than in the first (167), and it is almost negligible during third season; however, in all three rearingseasonsthe incidence ratio for SAL, CTE and RP remains nearly the same (60:30: 10). As with virosis, the mortality rate increases with age.

Transmission: Bacteriosis is transmitted orally. There is no possibility of transovarial transmission. After proper disinfection the layings yield healthy progenies.

Predisposing factors: Among the different factor inducing the disease, the feeding of over-mature leaves to early instar larvae is significant. Bacterial infection, unlike virosis, is accelerated by lower temperature and relative humidity.

3. Mycosis

Symptoms: The larva suffering from mycosis becomes hard, pale and inactive about 12 hours after infection. This is followed by a characteristic dorsal bending of the body. In 6 to 8 hours it dies, and within 48 hours the corpse is covered with green, powdery spores. The dead specimens ultimately become laterally compressed, dry, hard, brittle and mummified (Figure).



A. mylitta after death from mycosis

Causative agent: Two types of fungi, *Penicillium citrinum* and *Paecilomyces varioti,* are the causative agents of mycosis. The spore of *P. citrinum* is globular and greenish grey; that of *P. varioti* is oval and green. The *in vitro* life cycle of these two pathogens is 46 and 50 hours respectively (Figure).

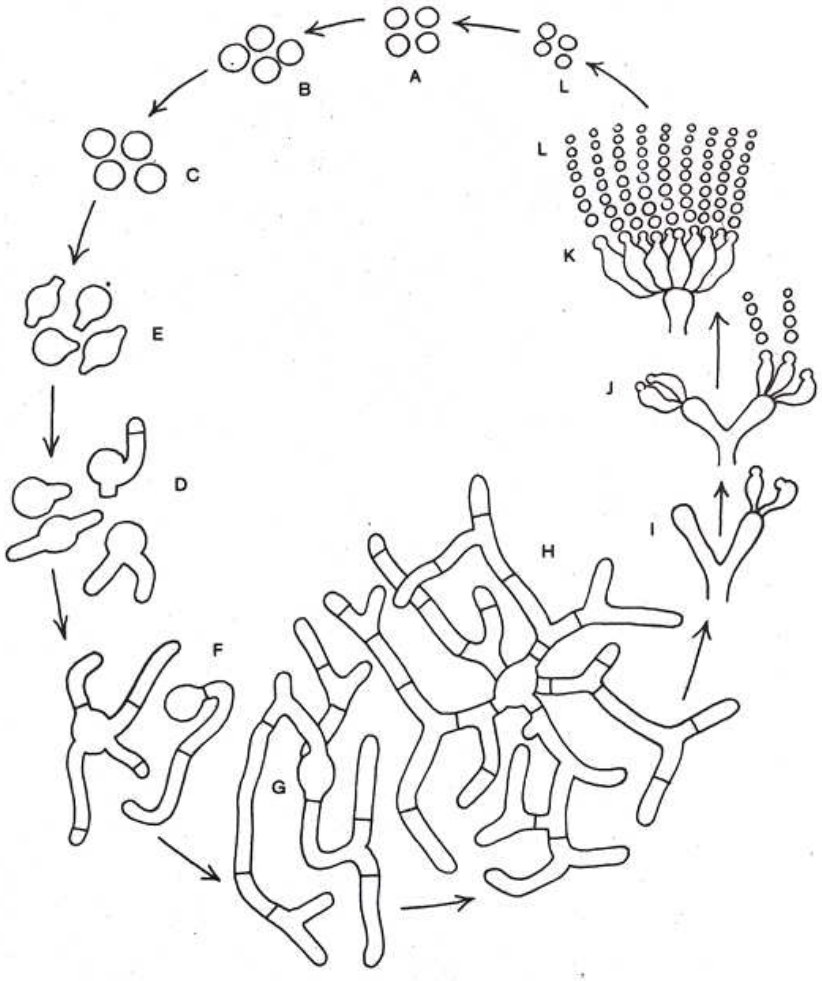


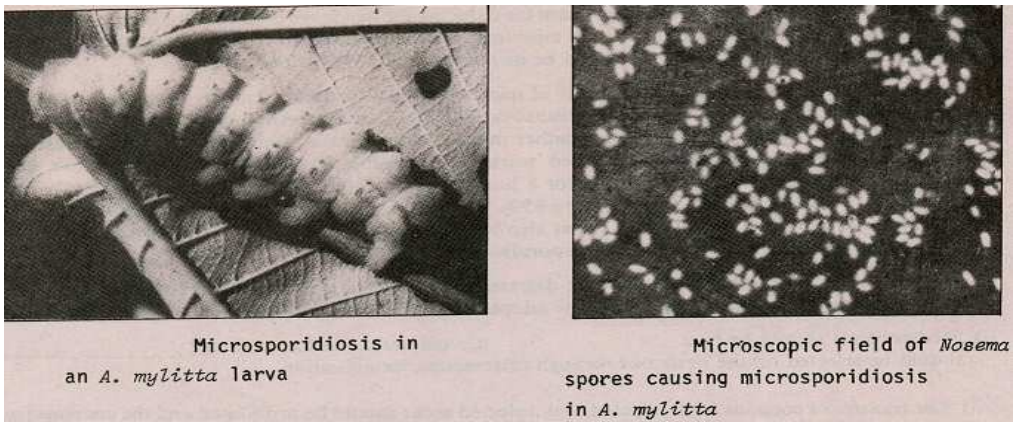
FIGURE 62. Development of *P. citrinum*
A, B, C - swelling of spores at 2, 6, 8 hours;
D - germination of spores at 10 hours;
E, F, G, H - development of vegetative bodies at 12, 14, 16, 18 hours;
I - development of fruiting bodies at 32 and 34 hours;
K - formation of conidiospores at 36 hours;
L - free conidiospores at 46 hours

Transmission: Mycosis is transmitted mainly through integumental injuries and the spiracles. After establishing itself on the integument of the larva, the spores germinate and radiate hyphal filaments which enter the body cavity and ramify inside the tissues. Attempts to reproduce the disease orally or transovarially have been unsuccessful.

Incidence: Mycosis appears early in the second rearing season (late August to early September); it is rare in the first rearing season and has not been observed in the third. The incidence, virtually nonexistent in the first two larval instars, occurs mainly in the third instar. The total loss is negligible.

Predisposing factors: Physico-physiological traumata, or wounds, and high humidity with a moderately high temperature are equally important contributing factors.

4. **Microsporidiosis** - Symptoms: Reduced feeding, sluggishness, a thin and darker body, black spots over the integument and stunted growth are the important symptoms of the disease (Figure). The black spots appear only after the second moult. The progenies of the infected brood exhibit disparity in larval growth and moulting.



Microsporidiosis in an *A. mylitta* larva

Microscopic field of *Nosema* spores causing microsporidiosis in *A. mylitta*

Almost 90 to 98% of the infected larvae die. Even those which survive are noticeably debilitated. The wings of infected adults are generally crumpled and their reproductive capacity is reduced. The scales become thin in density and adhere loosely.

Causative agent: The disease is caused by a monosporoblastic microsporidian of the family Nosematidae and the genus *Nosema.* The spore measures 5.80 + 1.16 microns in length and 2.3 ± 0.46 microns in breadth (Figure). The pathogen invades almost all the tissues, including

midgut epithelium, longitudinal muscle fibre, fat body, ovary, testis and haemocyte.

Transmission: The transmission of *Nosema* is oral, transovum and transovarial; however, transovum and transovarial transmissions are the most important modes of dissemination. The intensity of infection in the offspring directly correlates with that in the mother moth. The male moth cannot transmit the pathogen because the *Nosema* spore is bigger than the sperm head.

Controls: The chance of infection can be reduced substantially by precautionary measures, including microscopic examination of the mother moths, disinfection of eggs and maintenance of aseptic conditions during grainage and rearing. Microscopic examination is the most effective means of combating disease, especially if transmitted transovarially. For the preparation of disease-free layings a three-tier examination of the mother moths is recommended. Disinfection is also important for reducing the incidence of diseases. The eggs should be surface sterilized with 10% formalin for ten minutes and then followed by thoroughly washing in water; the rearing hut and equipment should be disinfected with 5% formalin.

Control of Microsporidiosis: The control of microsporidiosis remains a major problem in tropical tasar culture. As the disease is transmitted transovarially, the Pasteur method of preparing disease-free layings is the best remedy; however, it is rather impracticable on traditional tasar tracts because the layings are generally prepared by unskilled workers. A feasible alternative is the cumulative heat treatment, twice each at the pupal (40°C for 8 hours) and egg (460C for 5 minutes) stages. This treatment not only reduces infection by 80 to 85%, thus increasing the yield, but also helps improve the emergence and reproductive potential. It has also been observed that certain drugs, such as fumagillin and benomyl, are quite effective for microsporidiosis control.

Precautions in an epidemic: Of the four diseases of *A. mylitta* microsporidiosis is most fatal. The following precautionary measures should be adopted to combat the disease:

i) Seed cocoons should be procured only from uninfected areas (seed

zones), and disease free layings should be selected on the basis of thorough microscopic examination.

ii) The transfer of cocoons from infected to uninfected areas should be prohibitedand the cocoons from infected areas utilized solely for reeling.

iii) Smears of infected moths and larvae of infected lots, along with leaves pluckedfrom that particular bush, should be burned and buried.

iv) Periodic surveys should be conducted at both the larval and the pupal stage to forecast the possibility of epidemic.

2. PATHOLOGY OF ERI SILKWORM

Like mulberry silkworm, *eri* worms too are prone to several virulent and infectious diseases and pests. The germs and symptoms of diseases are more or less similar to those of mulberry silkworm diseases. Pebrine, flacherie and grassarie appear in this sector with varying degrees of virulence and infection. However, many of these diseases rarely cause damage if worms are kept under hygienic conditions and fed good quality leaves.

1. PEBRINE

Eri worms are subject to pebrine disease exactly as their mulberry counterparts are. The caustic organism of pebrine disease in *eri* worms belongs to genus *nosema.* The species, according to some, is not *bombycine.* It is reported that pebrine spores of mulberry do not cause pebrine in eri worm and vice-versa,

SYMPTOMS OF PEBRINE

Eri worms, suffering from pebrine disease, do not show any symptoms visible to naked eyes, until the disease is far advanced. The diseased larvae become more and more unequal in size, get sluggish and spin poor, flimsy cocoons. The moths emerge with difficulty and look deformed with small and scorched looking wings. They often become dull, black and lay eggs irregularly many of which do not hatch at all.

METHODS TO ELIMINATE PEBRINE

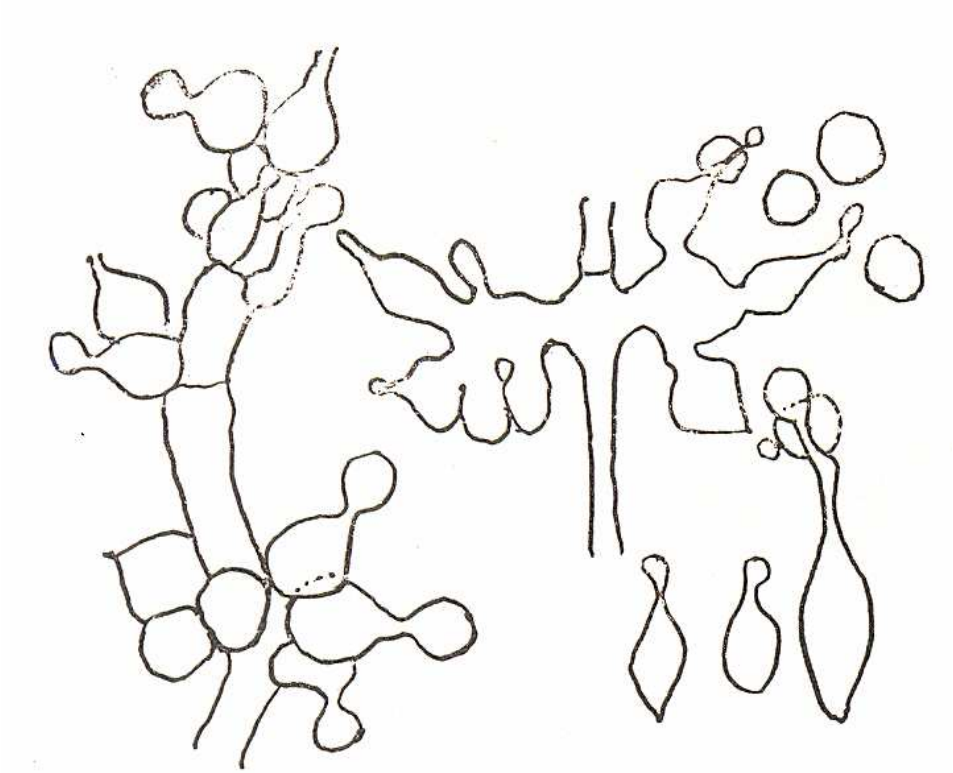
The disease can, however, be eliminated through appropriate measures. These measures include thorough disinfection of rearing rooms and washing of rearing appliances with germicides-2 to 4 % formaline and 2 % copper sulphate solution-avoidance of contamination and accumulation of dirt in rays etc. However, microscopical examination of moths and rejection of eggs laid by diseased ones are the best ways to eliminate pebrine disease from a lot.

2. MUSCARDINE

Attack of muscardine is not widespread in *eri* sector. The causal agent for this is a fungus: *Botrytis bassiana* which attacks other caterpillars besides *eri* silkworms. The fungus is reported to occur in association with other species of *Botrytis.* The worms attacked by muscardine become limp, lose elastic; cease to move and die rapidly. After death, the bodies become rigid and mummified. They do not soften and, very soon, become rotten. In a few hours, they usually get covered with a white efflorescence, as a result of which the body looks like a piece of chalk.

METHODS TO ELIMINATE MUSCARDINE

If careful watch IS kept on the worms under rearing, and the disease detected at the very outset, the worms can be saved. If any worm develops muscardine, it should be carefully picked up and placed in disinfectant solution. The rest of the worms should be immediately transferred to a clean tray with the help of a net. The discarded tray, alongwith refuse, should also be soaked in disinfectant water. However, other trays under rearings should not be stirred. Such cleaning should be continued till the fear of an outbreak disappears. Burning a small quantity of sulphur and maintaining light sulphur fumes in the rearing house at the rate of one oz. of sulphur, per 100 cubic yard of space, in addition to eliminating diseased worms as described above, would be helpful in checking the progress of disease. However, the disease in *eri* worms is not considered so very serious.



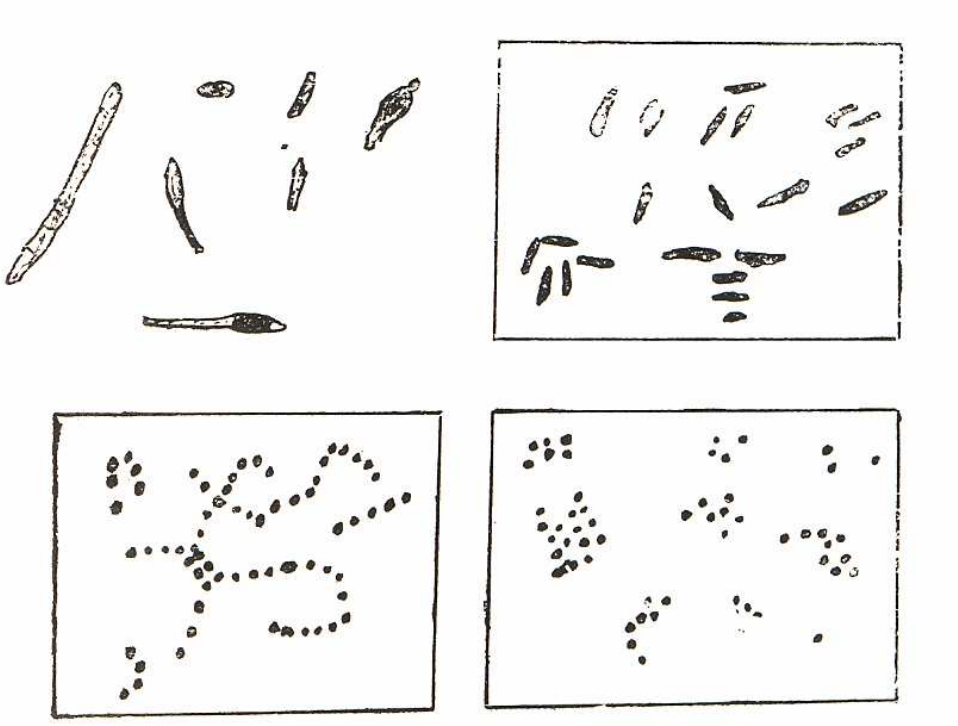
Muscardine

3. FLACHERIE

The disease, generally, occurs in grown up worms which die mostly just before spinning cocoons. They show characteristic symptoms. At first, they lose appetite and do not eat well. Soon, they look rather sickly losing the natural shine and healthy appearance of skin. The pellets of excreta become soft and stick to one another. The moults may not be shed properly as pieces of them remain stuck up around the body. With the progress of the disease, the worm becomes sluggish and vomits a brownish liquid. The faeces are soft and almost liquid which soil the anus. Finally, the diseased worm gets motionless, soft and discoloured. Soon thereafter, the body starts turning into black in the middle which, later, spreads over to the whole body putrefying and turning it into a liquid state. By now, it starts emitting a bad and sickly odour which is symptomatic only to flacherie.

The diseased and dead worms have a number of bacteria in their guts that cause fermentation and, later, putrefaction of the bodies. According to some, these bacteria are found in air, leaves and even in the guts of healthy

worms. But the present conclusions are that the disease is caused by some disturbances in the worm's metabolism or normal physiological functions and in the already diseased guts. They are, therefore, not a cause but the consequence of the disease. However, this disease is not considered infectious.



Flacherie

METHODS TO ELIMINATE FLACHERIE

High temperature, high humidity and bad ventilation are the causes of the flacherie disease. Other causes instrumental for flacherie are: dirty leaf, wet leaf, fermented leaf, coarse leaf, over feeding, over-crowding etc. To avoid the disease, fresh castor leaves should be given to the *eri* worms and fluctuations in temperatureof rearing room avoided..

4. GRASSERIE

Grasserie disease is caused due to excess of moisture in castor leaves. Worms affected by this disease show distinctive symptoms: becoming bloated in appearance, skin becoming somewhat shiny and yellowish in

colour and, the blood becoming turbid like pus instead of being clear as in ordinary worms. If the body fluid is examined under the microscope, it is found full of polyhedral crystalloids. They are also found in the different organs of the body. As they are brittle, they can be easily crushed by a little pressure on the cover slip on the glass slide.

The causal agent of disease, it is assumed, is some sort of a virus. The precautions to be taken are as follows:

1. Proper ventilation in the rearing house.
2. Avoidance of tender leaf in the case of grown up worms.
3. Feeding young-worms with tender leaf and mature worms with gradually matured leaf as they grow.

Both *P. ricini* and *P. cynthia* behave characteristically diverse to the grasserie disease-the former being immune and the latter receptive. Jucci (1954) failed to produce grasserie in *"eri* worms both by oral feeding and inoculation. But, the body of the moth frequently revealed the presence of abundant polyhedral corpuscles of identical nature of *Bombyx* (Valadares-1940). Hence, it is considered that the *eri* worm is a healthy carrier of virus. Steinhans (1949) refers that Bolle had experimentally infected the *P. cynthia* with the virus of silk Jaundice. Jucci also observed, sometimes after 1954, that in F 2 generation of the Cross of *cynthia* X *ricini,* some worms died of grasserie while the others developed normally.

3. PATHOLOGY OF MUGA SILKWORM

Muga silkworms are susceptible to various, diseases caused by protozoan, bacteria, virus and fungus. The intensity of the diseases varies during different seasons. Unless precautionary measures are taken the disease may eliminate the entire brood. The most important diseases of muga silkworms are discussed below

1. **Pebrine:** Pebrine is the most serious disease of muga silkworm caused by a protozoan, *Nosema* sp. The disease is transmitted from the infected mother 'moth to the offspring by transovarial means and this is generally called as primary infection. Also, the disease is transmitted to the healthy

larva by contamination. This is known as secondary infection. The infected larva becomes undernourished and lethargic. In mature larva, the silk glands are infected heavily and thereby a flimsy cocoon is formed. Pebrine infected larva or crysalis shows black dots or specks on the surface of the body and hence it is called as "phutuka" (spotted) in Assamese. If the spots are larger in size, it is known as "Hatiphutuka" and if small it is known as "Baliphutuka", When the infection is primary, most of the worms die in the second and third instar. If the infection is secondary, the worms may spin the cocoon of inferior quality.

The pebrine disease attacks muga silkworm during all the season and the infectivity is higher during autumn and winter seasons. The rearers do not use disease free certified seeds and hence muga 'crop loss due to pebrine is quite common. If the seeds are infected with pebrine, the hatching percentage of eggs is low, irregular growth of larvae is clearly visible and more than 50 per cent of the larvae die before third moult. Healthy larvae at fourth and fifth stages are also susceptible to pebrine due to secondary contamination, in such cases depending on the stage and severity of the infection the muga silkworm growth is affected. Infection during early fourth larval stage leads to formation of flimsy cocoon, whereas infection during fifth stage leads to vigorous growth of the larvae and produce well formed cocoons.

No control measure for the disease has yet been evolved. The elimination of diseased eggs during grainage by microscopic examination and diseased larvae during rearing are the only preventive measures against the disease.

2. **Flacherie:** Flacherie is called as "Mukhlaga" (evil mouthed) and caused by a virus which is followed by a secondary infection of bacteria. The incidence of the disease is high in summer months. Sudden fluctuations in temperature, bad weather, unsuitable leaf condition, high water content in-the leaf are the main causes for the virulence of the disease. The infected larvae become lethargic and motionless. The haemolymph of the heavily infected larva becomes black in colour. The larvae spin flimsy cocoon and die at the crysalis stage when the infection is at the last larval stage. This is

a very serious disease during Aherua and Bhodia (Summer) crops.

High atmospheric temperature and high humidity are favourable for the spread of this bacterial infection. During summer 20 to 30% larval mortality occurs due to flacherie. During winter season the infection of flacherie is considerably less (5-10%). Kotia and Jethua crops are comparatively free from this disease due to favourable climatic conditions.

Proper care of larvae at the early stage, protection of larvae from sun and rain, use of disease free layings and procurement of seed cocoons from the healthy zones are some, of the measures to minimise the incidence of flacherie disease.

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3. **Grasserie:** Grasserie is known as "Phularog" (swelling) and is caused by virus. The haemolymph of the infected larva turns milky and when examined under themicroscope numerous hexagonal crystals appear in the suspension. The incidence of the disease is high in summer when the atmosphere becomes highly humid. Grasserie is more common during. Aherua, Bhodia (summer) and Jarua (winter)cr9Ps. The crop loss due to the viral disease in these seasons varies from 20 to 25%. During summer and winter the climatic conditions are quite unfavourable and wide fluctuation in temperature within short period of time affects the growth and development of silkworm. Apart from climate, the quality of leaves also plays a vitalrole in the occurrence of grasserie disease. When muga silkworm in the fifth stage feeds on tender, succulent and diseased leaves, the incidence of grasserie has been observed more. Rearing of muga silkworm on tall and old trees should be avoided and bushy type dwarf plantations ensure removal of tender and succulent leaves and thereby reduces the incidence of grasserie to a greater extent.

Proper care during the rearing period, use of disease free layings etc. reduce theintensity of the disease in muga culture.

4. **Muscardine:** Muscardine is less prevalent in muga culture. The disease is caused by a fungus and hence the disinfection of dfls with 2% formaldehyde solution reduces the disease intensity by killing the fungal

spores which may adhere on the surface of the eggs.

Rectal protrusion: The rectal part of the alimentary canal of the larva comes out and the claspers loose the gripping power and fall down and die.

No control measure of the disease has yet been suggested. Research on different lines to control the intensity of the diseases by using antibiotics and anti-protozoan drugs are in progress.
