DISTRIBUTION AND MULTIPLICATION OF SYMBIOTIC MICROORGANISMS IN THE EGGS OF BROWN PLANTHOPPER DURING ITS EARLY EMBRYOGENESIS

G. SHANKAR and P. BASKARAN
Department of Entomology, Annamalai University, Annamalainagar 608 002, India.

THE existence and activities of symbiotic microbes are considered crucial for the normal nutritional and reproductive physiology of the host insect, particularly among Homopterans. It is well established that in host embryo the symbiotes multiply only to a limited extent and a mass increase is achieved only during post-embryonic development[1]. *Nilaparvata lugens* Stal, the brown planthopper (BPH), is an important pest in rice, known to harbour yeast-like organisms (YLOS) as symbiotes acquired transovarially[2]. The present report deals with the trend in multiplication of these symbiotes in the eggs of BPH from the time they are deposited to the external environment. Also the density of symbiote-inoculum was estimated in eggs before they were actually deposited.

To assess the level of inoculum of symbiotes in eggs before they were laid the abdomen of 10 gravid females was cut open and the eggs were carefully removed with a soft brush. The eggs were thoroughly rinsed in water twice to remove the adhering mycetocytes from the maternal tissues. The eggs were crushed individually in a cavity slide and the contents were thoroughly mixed with a drop of water. The yeast-like symbiotes were counted under a light microscope. Similarly the YLOS in normally laid eggs were counted at different time intervals.

In BPH the symbiotes are present as a small ball-like mass at the posterior pole of the egg. The diameter of the symbiotic ball was measured in 40 'undeposited eggs' and in 0.5–4 hr old normally laid eggs using a standard micrometer. The size of the symbiotic ball in the former category was 69.6 μ in diameter while it measured 82.8 μ in the eggs of 0.5 to 4 hr old indicating an active multiplication of the symbiotes within the period between two observations.

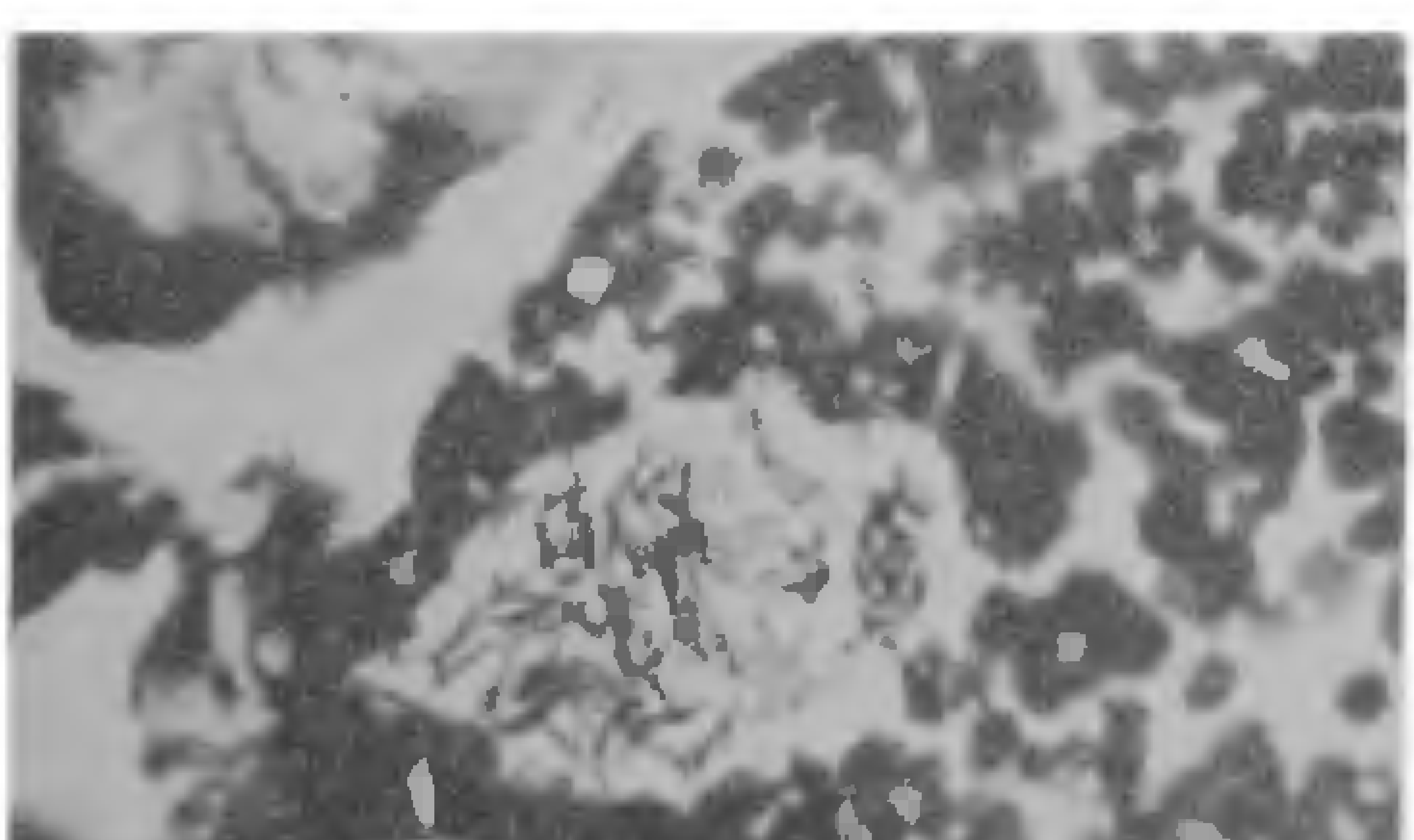

Figure 1. *Nilaparvata lugens* Stal. Primary oocyte showing the symbiotic-ball (SB) × 500

The eggs extracted from the insect's abdomen contained an average of 193.2 YLOS per egg while the mean number was 343.3 in eggs of 0.5 to 4 hr old. With the development of embryo the symbiotes gradually increased in number, the average being 787.8 in eggs of 60 to 65 hr and 1121.7 in eggs of 85 to 90 hr after oviposition. From this six-fold increase in the symbiotic population from its initial level it is evident that a state of equilibrium is maintained between the developing host embryo and its symbiotic population.

This low inoculum potential of symbiotes during early embryogenesis offers a possible stage exploitable to destabilise the host-symbiotic equilibrium through antibiotics aimed at the population control of the insect pest in the later stages.

One of the authors (GS) acknowledges financial support from ICAR, New Delhi.

10 April 1985; Revised 10 June 1985

1. Buchner, P., *Endosymbiosis of animals with plant microorganisms.* Interscience, New York, 1965
2. Nasu, S. and Suenaga, H., *Bull. Kyshu Agric. Expt. Stn.*, 1958, **5**, 71.

INDUCTION OF QUIESCENCE IN MUGA SILKWORM *ANTHERAEA ASSAMA* WESTWOOD

K. THANGAVELU, A. K. BHAGOWATI and A. K. CHAKRABORTY
Regional Muga Research Station, Central Silk Board, Mirza 781 125, India

MUGA Silkworm, *Antheraea assama* Westwood (Lepidoptera, saturniidae) is an endemic sericigenous insect of north-eastern India. It is multivoltine having five to six generations in a year. Spring and autumn seasons are more favourable for commercial rearing. Adverse environmental conditions such as high temperature and heavy rainfall in summer and low temperature and short day period in winter impose a major impediment for growth and development of the muga silkworm, resulting in uneconomical rearing. Yet, rearing has to be continued during these periods to maintain the race and augment the production of seeds for commercial purposes. The summer and winter rearing is known as seed broods. In recent years, increase in the intensity of pest attack[1] and physiological degeneration due to continuous inbreeding[2] have resulted in considerable reduction in seed production during seed broods. This has in turn posed a major problem for raising commercial crops contributing significantly to the current declining trend of muga silk production[3,4]. In order to resolve the problem, the present study was conducted to identify the response of muga silkworm to low temperature and explore the possibility of eliminating the unsuitable generations from rearing. Low temperature is known to arrest the development and induce quiescence diapause in many lepidopterans[5-7] including some sericigenous species[8-11].

Muga cocoons at three developmental stages, *viz* precocious larva (just prior to final ecdysis), pharate pupa (prepupa) and phaenerocephalic pupa (fully developed pupa) were used in the present experiment. During the winter of 1983–84 (Nov, Dec, Jan and Feb), 150 cocoons (50 each of precocious larva, pharate pupa and phaenerocephalic pupa) harvested from the commercial autumn rearing were exposed to prevailing temperature at high altitude (2,590 m above MSL.). Random samples were cut open and survival rate of larvae/pupae recorded once in a month. Prior to the onset of the immediate commercial rearing season in spring (March), the cocoons were brought to the plains (12.8 m MSL) and moth emergence, coupling, oviposition and hatching intensities were recorded (table 1). The larvae were reared on som (*Machilus bombycina* King) plants to determine the effective rate of rearing and shell ratio of cocoons.

In a second experiment 4,000 cocoons (1000 each precocious larva and pharate pupa and 2000 phaenerocephalic pupa) consisting of different age groups harvested from the spring rearing (June 1984) were subjected to low temperatures of 10°C and 5°C using cold storage. The exposure was made in a descending gradation to avoid cold shock to the larvae/pupae. The cocoons were exposed to normal temperature in an ascending gradation prior to the autumn generation and all parameters as in the previous experiment were studied (table 1). The data obtained were compared with those of normal in the corresponding periods. The sample size in the two experiments vary greatly and hence the results not compared between the experiments. Statistical analysis of both the experiments was done following Bailey[12].

The effect of low temperature varied with the developmental stage of the silkworm. Development in the precocious larva and pharate pupa was seriously impaired leading to total mortality within one month of exposure. In contrast, the phaenerocephalic pupa survived by entering into a protective quiescence as a fail-safe device at low temperature. The differential response of the phaenerocephalic pupa to low temperature is a reflection of its capacity for metabolic adaptation. Such programming is common among insects[5,13]. It is presumed that transient stages are more susceptible to environmental stress.

Quiescence did not have any deleterious effect on subsequent moth emergence, coupling, oviposition and hatching (table 1). The apparently lower number of eggs laid by the test females from high altitude was statistically insignificant ($p>0.05$). In all cases the effective rate of rearing was above 50% and no

Table 1 *The effect of low temperature preservation on muga silkworm*

Treatment	Moth emergence (%)	Coupling (%)	Eggs laid per female	Hatching (%)	Effective rearing rate (%)	Shell ratio (%)
High Altitude (2,590 m) (−2° to 14°C)	76	68	122 NS	84	52	8.1*
Control (12.8 m) (12° to 20° C)	79	62	183	75	59	7.1
(Cold storage) (12.8 m) 10° C	80	73	165	77	51	7.5
5° C	87	65	156	79	56	7.7
Control (12.8 m) (25° to 32°C)	82	68	158	77	61	7.3

* Significant at 1% level. NS – Not significant.

significant difference was noted. The shell ratio in the high altitude treated F_1 cocoons was 14.8% higher ($p < 0.01$) than that of the normal.

The induction of quiescence in the phaenerocephalic pupa has great significance to the muga silk industry. The hazardous rearing during summer and winter for seed purpose may be eliminated by this process. The surplus cocoons obtained from the spring and autumn generations can be safely preserved at low temperature which will serve as buffer stock between the two commercial generations. This may reduce the scarcity of seed cocoons thereby rejuvenating the muga silk industry.

7 February 1985; Revised 20 May 1985

1. Thangavelu, K. and Subba Rao, G., *Curr. Sci.*, 1982, **51**, 1023.
2. Subba Rao, G., *Natl. Sem. Silk Res. Dev.*, 1983, p. 11.
3. Anon., *Govt. of India Tariff Commission Report*, Govt. Press, Nasik, 1964, p. 63.
4. Anon., *Statistical Biennial*, Central Silk Board, Bangalore, 1984, p. 54.
5. Wigglesworth, V. B., *The principles of insect physiology*, 7th edn., Chapman and Hall Ltd., U.K., 1973, p. 746
6. Chapman, R. F., *The insects: Structure and function*, The English Language Book Society, London, 1973, p. 882.
7. Yagi, S., *The juvenile hormones*, Plenum Press, New York, 1976, p. 288.
8. Schneiderman, H. A. and Williams, C. M., *Biol. Bull.*, 1953, **105**, 320
9. Jolly, M. S., Sen, S. K. and Ahsan, M. M., *Tasar culture*, Ambika Publishers, Bombay, 1974, p. 176.
10. Sahey, D. N., Akhter, M. N. and Sinha, S. S., *Proc. 1st. Int. Sem. on Nonmulberry silk*, Ranchi, 1974, p. 170.
11. Jolly, M. S., Sonwalker, S. K. and Prasad, G. K., *Nonmulberry silks*, F. A. O., Rome, 1979, p. 164.
12. Bailey, N. T. T., *Statistical methods in biology*, English University Press Ltd., U.K., 1965, p. 152.
13. Denlinger, D. L., *Nature (London)*, 1974, **252**, 223.

HAEMOGRAM CHANGES DURING SENESCENCE PROCESSES IN *SCHIZODACTYLUS MONSTROSUS* D (ORTHOPTERA: SCHIZODACTYLIDAE).

SANJAY MANDAL and ANUPAMA MANDAL
Department of Zoology, A M College, Jhalda, Purulia 723 202, India.
**Department of Zoology, University of Burdwan, Burdwan 713 104, India.*

ALTERATION in the haemogram as an index of various physiological conditions in insects has been well studied[1-3]. Similarly, storage of different nutrients (carbohydrate, protein, lipid and amino acids) by haemocytes and their role in maintaining the normal nutrient balance during different stress conditions are also well documented[4,5]. Mandal[6] advocated that during the ageing process in *Schizodactylus monstrosus*, the volume of haemolymph and the number of haemocytes circulating in the haemolymph gradually decrease. He showed that some of the oxidative enzymes in haemolymph and fatbodies increase gradually with age. The present investigation reports the effect of a reductive agent on the changes of haemogram, total sugar, lipid, free amino acids and proteins in haemolymph with ageing process.

The source material used has been described elsewhere[7]. The stock was maintained in the laboratory at $28° \pm 1°C$ with 12:12 photoperiod and 90% relative humidity in sandy beds[8]. Adult insects were used for the experiment 2 hr after moulting. The insects were 5, 10, 20, 40, and 80 days old when sacrificed for haemocyte count and biochemical estimation. A 2% solution of the reduced glutathione in distilled water was injected at a dose of 40 μl insect after 2 hr of adult emergence. Control insects received only equivalent quantities of distilled water. Each insect was then labelled individually and separately kept in glass jars as the insects have cannibalic habits. Small nymphs of cockroach and grass-hopper were given as food[7].

Haemolymph was collected from both control and treated insects by puncturing the femur of the hind leg using a graduated glass capillary tube and kept in a test tube at $4\ C \pm 0.5\ C$ until ready for use. Total sugar in the haemolymph was determined following the anthrone method[9] using glucose as the standard. Total lipid was estimated by vanilline reactive method[10]. Free amino acid was estimated spectrophotometrically[11]. The total protein content was estimated by standard technique[12]. The pH of the haemolymph was determined using microindicator pH paper. The speci-