Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 98, No. 1, January 1989, pp. 27–33.

Effect of cold storage of newly hatched larvae on survival rate, growth and egg production in silkworm *Bombyx mori* L.

K V BENCHAMIN, VIJAYALAKSHMI RAO and
P JAYARAMA RAJU
Central Sericultural Research and Training Institute, Manandavadi Road, Srirampura, Mysore 570 008, India

MS received 6 April 1988; revised 5 December 1988

Abstract. Effects of refrigeration (5 ± 1°C) of newly hatched silkworm larvae on survival rate, growth and egg production were studied in two multivoltine races, Pure Mysore and Hosa Mysore. When refrigerated for 10 days, moth emergence (males: 83·3% vs 89·4% and females: 92% vs 90·2%) and fecundity (465·5 vs 458·3) did not differ significantly from the control. Reductions in cocoon weight by 4% (1·323 g vs 1·271 g) and 10·7% (1·323 g vs 1·181 g) were caused by 5 and 10 days of refrigeration. Marked increase in early stage (I and II instar), loss of larvae (12·9% vs 48·8%), decrease in effective rate of rearing (77·1% vs 33·5%), pupation rate (92·4% vs 65%) and reproductive rate (137·7 vs 36·9) were observed, when refrigerated for 10 days. Results on reproductive rate indicated one day's refrigeration as safe period in Hosa Mysore but not in Pure Mysore. In December season, both races showed higher tolerance and possibility of safe refrigeration upto 2 days.

Keywords. *Bombyx mori*; early stage loss; effective rate of rearing; pupation rate; reproductive rate; egg production.

1. Introduction

Refrigeration of cocoon or pupa, moth, egg and larva at one or more stages are followed to synchronise silkworm rearing with the availability of mulberry leaf. In silkworm seed production, refrigeration of seed cocoon and moth are inevitable to adjust adult emergence or effect mating of desired parents. Usually, refrigeration of cocoon is limited to 1 to 2 days, though the recommendations are up to 3 days for female and 7 days for male pupa (Tazima 1962; Jolly 1983). In case of moths, it is limited to 3 days in female and 7 days in male (Ullal and Narasimhanna 1978; Jolly 1983). The efficiency of mating of male moths was improved, when kept at 5 ± 2°C (Subramanyam 1982). Bivoltine eggs, characterised by hibernation, are refrigerated at 2·5°–7·5°C for different period to activate them to hatch at the desired time (Mizuno 1920; Watanabe 1931; Katsukake 1952). The non-hibernating multivoltine eggs can be cold stored on second day at 5°–7°C to postpone hatching up to 24 days (Dutta *et al* 1972). Eggs at blue body stage can be refrigerated for 2 to 3 days at 5°C to delay hatching (Tanaka 1964). Refrigeration of newly hatched silkworm larvae is also not uncommon, especially when hatching is irregular and a single brushing is desired. However, unlike other stages of pupa, moth and egg, information on refrigeration of hatched larva and subsequent rearing performances are limited though not totally lacking (Jolly 1958, 1983; Tazima 1962; Tanaka 1964). The present study is carried out to understand more about the effect of refrigeration of newly hatched silkworm larvae on their rearing performances and reproductive rate.

2. Materials and methods

Two multivoltine races, Pure Mysore (PM) and Hosa Mysore (HM) were used. Newly hatched larvae refrigerated for 1–10 days and the control without refrigeration formed different treatments. Refrigeration was done at 5±1°C and RH 75±5%, in a commercial silkworm seed cold storage. Hatched larvae, continuously for 10 days from freshly prepared layings for each day, were refrigerated and were released on a single day to facilitate brushing and rearing at a time. Each treatment consisted of 3 cellular replications. Rearing was conducted as per standard recommendations (Krishnaswami *et al* 1973). Test was repeated in 3 distinct seasons [June–July 1986 (S_1), August–September 1986 (S_2) and December 1986 (S_3)], to concur the results.

Different parameters studied were lost during early instar (I and II) rearing (early stage loss = ESL), effective rate of rearing (ERR), cocoon weight, pupation rate (PR), female and male moth emergence, fecundity and reproductive rate (RR). The definition of these terms and calculations are interpretable as given in standard sericulture text books (Krishnaswami *et al* 1973; Ullal and Narasimhanna 1978; Narasimhanna 1988) and related publications (Krishnaswami 1978, 1979; Benchamin and Krishnaswami 1981a, b). Analysis of variance (3 way factorial) was carried out for interpretation of results.

3. Results and discussion

3.1 *Survival rate*

In the present study, all treatments recorded significant increase in ESL of worms compared to control and it increased with the increased days of refrigeration (table 1). The ERR was reduced significantly in all treatments, when refrigerated for 10 days. ESL increased from 12·9–48·8% and ERR decreased from 77·1–33·5%. Inability to feed and moult normally, resulting in irregular growth, were characteristic of larvae refrigerated for more than 5 days in both the test races. Those larvae that survived in these treatments were also prone to viral and bacterial diseases in later instars. Starved larvae in silkworm *Bombyx mori* are more susceptible to flacherie (bacterial disease) and grasserie (viral disease) (Samson *et al* 1981). In the present study, even refrigeration for 1 day caused 18·6% increase in ESL (12·9% vs 15·3%) and 7·1% reduction in ERR (77·1% vs 71·6%).

Significant races × treatments and races × seasons interaction (table 6) showed specific advantages of HM race over PM and S_3 season over other seasons. For example ESL in 1 day refrigerated batch and ERR up to 2 days refrigeration in HM, were not significantly different from the control. In PM, increase in ESL and reduction in ERR were significant in all treatments (table 2). In respect of ESL and ERR, 2 days refrigeration was found safe in S_3 season.

3.2 *Growth*

Cocoon weight is directly correlated to the larval body weight. It reduced significantly in all treatments when compared to control, but did not vary significantly

Table 1. Effect of refrigeration (5 ± 1°C) of newly hatched larvae on various characters studied (mean of 3 seasons).

Refrigeration (days)	Larval loss in early instars (%)	Effective rate of rearing (%)	Cocoon weight (g)	Pupation rate (%)	Female emergence (%)	Male emergence (%)	Fecundity (No)	Reproductive rate (No)
Control	12·9 (17·88)	77·1 (61·89)	1·323	92·4 (73·97)	92·0 (73·80)	88·3 (70·79)	465·5	137·7
1	15·3 (22·46)	71·6 (58·06)	1·281	89·2 (71·40)	90·4 (72·14)	90·2 (71·85)	460·1	121·1
2	17·9 (24·78)	68·0 (54·85)	1·285	87·7 (70·92)	89·9 (71·65)	87·3 (69·79)	464·6	112·7
3	25·0 (28·45)	59·7 (52·39)	1·277	83·7 (68·77)	89·4 (71·01)	88·0 (70·32)	459·5	102·6
4	27·3 (32·62)	58·5 (48·29)	1·261	85·5 (68·38)	89·8 (71·49)	86·0 (69·21)	463·0	91·8
5	31·1 (33·49)	53·4 (47·37)	1·271	80·4 (65·26)	89·8 (71·49)	88·5 (71·51)	463·5	83·6
6	33·2 (34·61)	51·3 (44·88)	1·228	80·5 (64·97)	90·3 (71·98)	88·4 (70·54)	458·5	80·2
7	33·4 (35·73)	45·3 (41·78)	1·237	72·3 (60·77)	90·9 (72·56)	88·2 (71·28)	456·5	63·0
8	41·2 (40·68)	41·2 (39·43)	1·235	76·3 (59·06)	90·5 (71·31)	86·6 (69·61)	460·0	54·9
9	50·0 (44·96)	37·6 (37·48)	1·245	73·3 (58·58)	92·0 (73·72)	87·9 (70·64)	461·3	47·5
10	48·8 (45·96)	33·5 (34·67)	1·181	65·0 (52·62)	90·2 (72·09)	89·4 (71·98)	458·3	36·9
SE	± 0·788	± 0·745	± 0·009	± 0·898	± 0·640	NS	NS	± 2·44
CD at 5%	2·21	2·09	0·026	2·51	1·79			6·9

Numbers in parentheses are transformed values.

Table 2. Early stage loss (%) and ERR (%) influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	Early stage loss (%)*		ERR (%)*	
	PM	HM	PM	HM
Control	17·57	18·20	63·23	60·56
1	26·21	18·71	55·79	60·33
2	26·59	22·98	51·60	58·11
3	29·61	28·28	50·69	54·08
4	32·71	32·54	47·49	49·09
5	33·62	33·56	46·83	47·91
6	35·01	34·20	42·18	47·59
7	35·54	35·92	39·92	43·65
8	39·63	41·72	40·07	38·78
9	42·85	47·06	39·94	35·03
10	43·72	48·14	36·82	32·52
SE ±	1·114		1·054	
CD at 5%	3·12		2·95	

*Transformed values.

Table 3. Cocoon weight (g) influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	Races		Seasons		
	PM	HM	S_1	S_2	S_3
Control	1·133	1·513	1·451	1·103	1·416
1	1·055	1·506	1·410	1·063	1·369
2	1·056	1·514	1·386	1·107	1·362
3	1·002	1·552	1·419	1·052	1·360
4	1·032	1·489	1·417	1·025	1·341
5	1·005	1·537	1·444	1·032	1·336
6	0·965	1·491	1·308	1·048	1·329
7	0·969	1·504	1·339	1·045	1·326
8	0·970	1·501	1·351	1·041	1·314
9	0·984	1·505	1·370	1·038	1·320
10	0·930	1·436	1·266	0·964	1·320
SE ±	0·013			0·016	
CD at 5%	0·037			0·045	

among the first 5 days and also among 6–9 days of refrigeration (table 1). Results indicated that growth in those larvae that survived and formed cocoons, was more or less same and it was affected at lesser degree compared to ESL and ERR. This is more clear in seasons S_2 and S_3 over S_1 and in race HM over PM, where cocoon weight was not affected among treatments of 1–9 days refrigeration (table 3).

3.3 *Egg production*

Pupation rate, female and male emergence and fecundity were the characters studied, as factors contributing to egg production. The reproductive rate, expressed

as multiplication rate in earlier studies (Benchamin and Krishnaswami 1981a, b), was also assessed as an index to compare the egg production efficiency in different treatments. Among these, male moth emergence and fecundity were not affected significantly by refrigeration (table 1). Female moth emergence was also not influenced, as the differences among treatments and between many treatments and control were not significant. PR differed significantly in all treatments compared to control and it steadily decreased with the increase in refrigeration days, from 92·4% in control to 65% in 10 days refrigeration. Race HM showed better tolerance to the treatment, since PR remained not significantly ($P<0·01$) different from the control,

Table 4. Pupation rate (%)* influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	Races		Seasons		
	PM	HM	S_1	S_2	S_3
Control	74·83	73·10	74·25	76·06	71·59
1	70·26	72·54	72·73	71·01	70·42
2	69·29	72·55	67·39	73·94	71·41
3	64·49	73·04	67·50	69·69	69·11
4	65·96	70·80	65·27	70·34	69·63
5	62·20	68·30	61·77	68·57	65·43
6	60·59	69·37	59·85	67·92	67·17
7	56·56	61·56	50·51	62·22	64·45
8	59·81	61·78	51·31	67·23	63·85
9	58·56	58·59	45·87	65·59	64·28
10	53·14	52·11	39·99	58·99	58·88
SE ±	1·269			1·555	
CD at 5%	3·56			4·35	

*Transformed values.

Table 5. Reproductive rate influenced by refrigeration (5 ± 1°C) of newly hatched larvae.

Refrigeration (days)	PM				HM			
	Seasons				Seasons			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
Control	113·62	125·63	126·80[a]	122·02	149·86[b]	139·08[c]	171·29[d]	153·41[e]
1	77·17	98·97	111·04[a]	95·73	149·63[b]	128·96[c]	160·86[d]	146·48[e]
2	56·28	76·65	116·36[a]	83·09	136·27[b]	133·31[c]	157·20[d]	142·26
3	50·86	76·31	105·74	77·64	123·55	106·76	152·21	127·51
4	52·67	71·40	101·64	75·24	106·27	94·23	124·66	108·33
5	39·10	73·25	89·52	67·29	103·68	87·11	108·92	99·90
6	35·59	41·45	93·94	56·99	104·77	89·56	115·66	103·33
7	28·76	31·04	82·77	47·53	65·87	68·73	100·67	78·44
8	27·83	30·30	75·96	44·69	59·21	36·85	99·38	65·15
9	29·01	35·05	68·16	44·07	39·07	24·45	88·93	50·82
10	16·63	26·07	60·88	34·53	20·99	21·08	75·95	39·34
SE ±		5·99		3·46		5·99		3·46
CD at 5%		16·80		9·70		16·80		9·70

Treatments indicated by same letter are not significantly different from control.

Table 6. Analysis of variance.

Source of variation	df	Larval loss in early instars	Effective rate of rearing	Cocoon weight	Pupation rate	Female emergence	Male emergence	Fecundity	Reproductive rate
Race (R)	1	NS	**	**	**	**	NS	**	**
Seasons (S)	2	**	**	**	**	NS	NS	**	**
Ref. days (D)	10	**	**	**	**	**	NS	NS	**
R × D	10	**	**	**	**	NS	NS	NS	**
R × S	2	*	**	**	**	**	NS	**	**
D × S	20	**	**	**	**	NS	NS	NS	**
R × S × D	20	**	NS	*	**	NS	NS	NS	**
Error	132								

NS, Not significant, $*P<0.05$ and $**P<0.01$.

up to 4 days of refrigeration. The same was true in S_3 season over S_1 and S_2 seasons (table 4).

Reproductive rate was affected significantly ($P<0.01$) in all treatments, compared to control (table 1). However, racewise comparison showed that newly hatched larvae could be refrigerated in HM, for one day, without causing significant reduction in RR (table 5). In PM, there was no scope for refrigeration of larvae, as it reduced RR significantly. Seasons x treatments interaction showed 2 days refrigeration in S_3 season in PM and in all seasons in HM, was safe, as the differences in RR compared to control, were not significant. Refrigeration of newly hatched larvae for 5 days resulted in 33·3% and 34·9% reduction in RR in PM and HM, respectively. When refrigerated for 10 days, the corresponding reductions were 71·7% in PM and 74·4% in HM. Since fecundity and male moth emergence were not significantly different from the control and in respect of female moth emergence and cocoon weight the effects were of lesser degree, the significant reduction in RR in different treatments was mainly caused by poor survival and pupation rate.

Acknowledgements

Thanks are due to Dr Manjeet S Jolly for his keen interest in the present work and constant encouragement, Mrs Kshama Giridhar for statistical analysis of data and staff of Seed Technology Laboratory for their help in conducting silkworm rearing.

References

Benchamin K V and Krishnaswami S 1981a Studies on the egg production efficiency in silkworm *Bombyx mori* L. I; On the various factors contributing to egg production; *Proceedings of the Sericulture Symposium and Seminar*, Tamil Nadu Agricultural University, Coimbatore, pp 1–6

Benchamin K V and Krishnaswami S 1981b Studies on the egg production efficiency in silkworm *Bombyx mori* L. II; Egg production efficiency in hybrid parents and its effective application; *Proceedings of the Sericulture Symposium and Seminar*, Tamil Nadu Agricultural University, Coimbatore, pp 7–14

Dutta R K, Sengupta K and Biswas S N 1972 Studies on the preservation of multivoltine silkworm eggs at 5–7 ± 1°C; *Indian J. Seric.* **11** 20–27

Jolly M S 1958 Resistance to starvation of the newly hatched silkworm; *Central Silk Board Newslett.* **3** 10–11

Jolly M S 1983 *Organisation of Industrial Bivoltine Grainage for Tropics*, (Bangalore: Central Silk Board)

Krishnaswami S, Narasimhanna M N, Suryanarayana S K and Kumararaj S 1973 Sericulture Manual-2 Silkworm Rearing; *CSRTI Mysore Bull.* **2** 1–23

Krishnaswami S 1978 New technology of silkworm rearing; *CSRTI Mysore Bull.* **2** 1–23

Krishnaswami S 1979 Improved method of rearing young age (Chawki) Silkworms; *CSRTI Mysore Bull.* **3** 1–24

Kutsukake H 1952 *Studies on the preservation of the Silkworm eggs*; *Special Report* (Japan: Kanebo Sericult. Exp. Sta.)

Mizuno T 1920 On the cold storage of silkworm eggs; *Sanshi Shikenjo Hokoku* **4** 205–262

Narasimhanna M N 1988 *Manual on silkworm egg production* (Bangalore: Central Silk Board)

Samson M V, Nataraju B, Baig M and Krishnaswami S 1981 Starvation of *Bombyx mori* L. on cocoon crop and incidence of loss due to diseases; *Proceedings of the Sericulture Symposium and Seminar*, Tamil Nadu Agricultural University, Coimbatore, pp 163–167

Subramanyam K 1982 *Studies on the effect of rest on the efficiency of male moths in relation to number of matings and occurrence of viable eggs in Silkworm Bombyx mori* L, STS dissertation, CSR and TI, Mysore

Tanaka Y 1964 *Sericology* (Bangalore: Central Silk Board)

Tazima Y 1962 *Silkworm egg* (Bangalore: Central Silk Board)

Ullal S R and Narasimhanna M N 1978 *Handbook of practical Sericulture*; (Bangalore: Central Silk Board)

Watanabe K 1931 Relation between the temperature after oviposition and the diapause of silkworm eggs; *Sanshi Shikenjo Hokaku Tech. Bull.* **41** 16–30