慶北大農學誌(Agric. Res. Bull. Kyungpook Natl. Univ.) 18(2000. 12) 43~51

Study on the effect of different temperatures on the main productive characters of the silkworm larvae, *Bombyx mori* under Egyptian agro-climatic conditions

H. Greiss, H.R.Sohn* and J.S. Lim*

Agricultural company Agro Mier,9 Malek ,El- Afdal Street 11211 Zamalek, Cairo Egypt
**Department of Natural Fiber Science, College of Agriculture, Kyungpook National University,Taegu,Republic of Korea*

에집트 기후조건 특히 온도의 변화가 원원잠품종의 주요 유전형질에 미치는 영향

H. Greiss · 손해룡* · 임종성*

*경북대학교 농과대학 천연섬유학과

Abstract

Eleven inbred lines of silkworm, Bombyx mori L. were exposed to two main different temperature (23C and 25C) during the larval period in order to elucidate the effect of the temperature differences on their main characters under the, prevailing in Egyptial agro-climactic conditions.

The results show that the temperature differences did not affect significantly the pupation ratio and cocoon shell ratio. However, they affected their larval duration to become shorter with the high temperature treatment (27℃) and their cocoon shell weight and cocoon yield per box of silkworm eggs to be higher in the lower temperature(23℃).

It was recommended that some inbred lines should be bred at the 23C throughout the whole larval period, particularly in being reared with high nutritional leaves in order to maximize the hybridization of cocoon yields.

Key words : silkworm, *Bombyx mori* L., pure breeds, temperature, nutrition

Introduction

Sericulture development activities into Egypt were initiated in the eighteenth century as mulberry cultivation and silkworm rearing were introduced in to the country. The number of farmers involved in sericulture in Egypt is estimated at approximately 3000-3500 and more than 50 percent of them are women who are involved not only in cocoon production activities, but also in hand reeling and carpet manufacturing.

Mulberry cultivation for cocoon production is considered as one of the most profitable crops in the country in terms of their earning in comparison to other agricultural crops. However, the country should import annually about 50 tons of inferior quality yarns.

To meet the high demand of silk-yarn in Egypt and to substitute the importation with the local productions, there is a need to increase the silk production. As Egypt is located in the subtropical zone, tropical mulberry varieties such as Canva-2 sprout throughout the year and it is possible to try multiple rearing in the year. However one of the problems in the multiple rearing is the control of temperature and its effect on the silkworm development and cocoon productivity.

The body temperature of the silkworm larva varies with ambient temperature (Reali *et al.*,1990) as well as it influences the physiological activities, food intake and economic parameters(Tzenov and Maldenov, 1996; Muniraju *et al.*,1999).The effect of temperature during rearing on survival, growth, cocoon production and silk quality have been studied by many workers (Matsumura *et al.*,1958; Ueda,1963; Ueda and Marizuka, 1962; Yakomoto and Fujimaki,1962; Verma and Atwal,1967; Sigematsu and Takeshita,1967; Rapusas and Gabriel,1976; Muniraju 1995). The effect of the temperature on the silkworm, Bombyx mori L.depends on a number of factors such as the stage of metamorphosis, genotype, nutrition, humidity, and air velocity (Petkov,1972; Karaivanov, 1989; Takagishiand Ueda,1991; Manjula,1990; Krishnaswamy,1992; Shirota, 1992). Singh *et al.* (1998) reported that silkworm being a poikilotherm, its body temperature changes according to the environmental temperature. The infant silkworms require a comparatively higher temperature and the amount of mulberry leaf ingestion, digestion and metabolic rate are very high in young instars owing to the high temperature provided.

Jolly (1987) and Ullal ad Narisimhana (1987) reported that the early instar larvae are resistant to high temperature 26-28℃ and it is also favorable for healthy growth of them. Tzenov (1996) studied the effect of low (18℃), moderate (23℃) and high (28℃) temperatures on the dry matter in food during the fifth instar in 2 Bombyx mori pure lines, designated as Super1 and Hessa2 having Japanese and Chinese origins respectively. The results obtained indicated that low temperature at the fifth larval instar rearing increased the quantities of food ingested and digested, and also the food digestibility. However, high temperature manifested the opposite effect and the food digested was utilized most effectively under the high temperature than in the low and moderate temperature. Taking in consideration the high influence of the temperature on the silkworm, Bombyx mori L. during the larval stages of its metamorphosis, it is necessary to detect the suitable thermo-regime for the different highly productive races when they are fed with quality mulberry leaves.

The present study has been carried out to analyse in detail the effect of two different temperatures during young and late instars on the main productive character values under the specific Egyptian conditions.

Materials and methods

The experiments were conducted in Agromier Co. mulberry plantation and rearing houses located in Asyut province in Egypt 320 km south of Cairo in a desert area during the period 1997-1998. The plantation was irrigated by the river Nile water regularly at the plant needs, as there are no rains in this area. Mulberry plantation is mainly of Kokuso 27 and Canva 2 varieties, shaped in bush type and pruned in fist form after 3 step up branch harvests every year.

Fertilizers applied to the mulberry garden:

Nitrogen(N),Phosphorus(P)and Potassium(K) at dose of 300:150:120 units per hectare were applied in addition to secondary and micronutrients.

As secondary nutrients we added calcium (Ca) and sulphur (S) within macro-fertilizers (NPK) as well as magnesium that is added with micronutrients.

The recipe for preparing the mixture of micronutrients was as same as Table 1.

Table 1. Amount of Salts*Added

Salt	Symbol	Amount	Concentration
Magnesium Sulphate**	MgSO4	50kg/ha	Mg 11%
Manganese Sulphate	MnSO4	30kg/ha	Mn 27%
Ferrous Sulphate	FeSO4	10kg/ha	Fe 19%
Zinc Sulphate	ZnSO4	10kg/ha	Zn 35%
Copper Sulphate	CuSO4	1.5kg/ha	Cu 28%
Boric Acid	H3BO4	2kg/ha	B 17%
Ammonium Molybdate	(NH3)2MooO4	1kg/ha	Moo 54%

*The author previously tested the amounts and the percentage between the nutrients.
**Magnesium is a secondary nutrient.

The fertilizers were uniformly added in trenches after each branch harvest, just before 3rd irrigation. Rearing houses were equipped with automatic temperature and humidity control devices. In the control groups the rearing was done at standard regimes of temperature and humidity(Table 2) as described in Japanese, Korean, Indian, Bulgarian and Ukrainian literatures(Petkov *et al.*, 1989; Ajuzawa, 1971; Lim. *et al.*, 1990; Krishnaswamy, 1993; Zlotin and Bolavin,1988).

Table 2. Temperature and humidity for standard rearing

Age	Temp.	Humidity	Light	Air speed
1&2 instars	27℃	85% RH	25 Lux	0.1 m/sec
3 instar	25℃	80% RH	25 Lux	0.1 m/sec
4&5 instars	23℃	70% RH	25 Lux	0.3 m/sec
Mounting & spinning	27℃	60% RH	25 Lux	0.5 m/sec

Rearing was performed using plastic trays in size of 70cm x 40cm x 30cm (LxWxH) accommodating 100 larvae counted just after 2nd moult. During each experiment all the larvae were fed by equal amounts of leaves of the same variety. Bed spacing was performed every day before morning feeding, and bed cleaning was performed once after each moult. Bottle brush type plastic mountages were used for mounting larvae. Eleven breeds were used throughout the experiments and each breed was tested in 4 replicates of 100 larvae counted immediately after the 2nd moult. Breeds were obtained from the AGROMIER germ bank and they represented 3 Chinese, 3 Japanese, 3 European and 2 egg sex-limited breeds. All breeds were from different geographical origins, bred and selected in AGROMIER germ bank and maintained under these codes. We implemented house disinfections by chlorinated lime (Bleaching powder) solution in 5% W/V of CaOCl2. Next day acetic acid solution 0.2% of CH3COOH. Also we made egg disinfections by acetic acid in 0.04% for15 min. at room temperature. Bed and larvae disinfections were performed after every moult when 95% of larvae finished their moulting, and a special powder for bed disinfections was applied uniformly at the rate of 250 gm/m^2 of bed in young instars and 500 gm/m^2 at late instars. Powder was applied once on the 4th day of the 5th instar before the 1st morning feed when there were no edible leaves in the beds. The bed disinfectant was prepared by mixing Calcium Oxide (88%), Bleaching powder(10%), Benzoic acid(1%) and Diathane M45(1%).

Different temperature regime: We formed the following experimental groups.

Experiment 1; All instars reared at 23℃

Experiment 2; All instars reared at 27℃.

Experiment 3; First till 3^{rd} instars reared at 23℃, 4^{th} & 5^{th} instars reared at 27℃

During moulting the humidity was reduced to 50% RH and nylon cover was removed from the beds of the young instar larvae maintaining the original temperature. We fixed Intensity of light, relative humidity and air speed in control and experiments. During two years 1997 and 1998, 4 trial rearings a year of all breeds were conducted in 4 replicates of 100 larvae each, and the cocoon collected were tested for the main productive character values. The data were statistically analysed using the standard methods.

Results

From table 3 it is evident that temperature regimes gave the same influence on the tested breeds irrespective of their origins. The average data in 2 years manifested no effect of the rearing temperature of different regimes on the pupation ratio. It means that temperatures in range of 23-27℃ are optimum for normal development of silkworm larvae and accordingly to manifest high survival ratio. Naniraju *et al*(1999) reared the silkworm races Pure Mysore (multivoltine) and NB4D2 (bivoltine) under constant temperature rearing of 26, 28, 30 and 32℃ during young and late ages with all different combinations. He reported that low temperature 26℃ throughout the rearing favored the higher silk conversion with better survival in bivoltine with 87% of survival ratio , and our results confirmed his finding with even a lower temperature i.e. 23C. Our results showed one-day extension of larval period in Experiment 1 and one-day shotening in Experiments 2 and 3 compared with the control. Other authors Venugopalpilli and Krishnaswamy (1987), Hanumappa (1988) and Bashkar *et al* (1992) detected a direct correlation between metabolic responses of the poikilotherm Bombyx mori to temperature. This is in confirmation to the results obtained by us in this study.

The data for the cocoon weight and cocoon shell weight manifested that most of the tested Breeds showed a significant increase in both characters in Experiment 1 , a decrease in Experiment 2 and no significant change in Experiment 3 compared to the control. The highest and proved values in cocoon weight are demonstrated in Experiment 1 that exceeds Experiments 2, 3 and the control by 0.156-0.461(11.22-23.04%)gm, 0.107-0.257(5.18-11.660%)gm and 0.036-0.135(2.99-6.14%)gm, respectively. Analogically the highest values for shell weight were recorded in Experiment 1 and they exceeded Experiment 2, 3 and the control by 0.033-0.098gm (7.43-21.26%), 0.020-0.055gm (4.18-11.53%) and 0.012-0.035gm (2.97-7.04%) respectively. As a rule, the breeds with Japanese origins obtained comparatively higher increase in shell weight in Experiment 1 followed by the breeds with Chinese origins. According to Tzenov (1996) and Tzenov and Maldenov (1996) low temperature (18℃) and moderate (23℃)in fifth larval instar rearing increased the quantities of food ingested and digested, and also the food digestibility, but high temperature manifested the opposite effect. Low temperature in 5th instar larvae rearing also increased the cocoon weight, shell weight and cocoon yield. It also increased moth emergence percentage and the number as well as the weight of eggs per laying. Also Ueda *et al* (1969) reported that there were not any significant differences in the growth rate, cocoon weight, shell weight and fecundity between rearings when the silkworms were reared at 22 and 26℃ during the 5th instar and the larvae were fed by good quality mulberry leaves. Ueda and Suzuki (1967), Junliang and Xiaofeng (1992) and Muniraju (1995) reported that the increase in the temperature from 20 to 30℃ decreased the conversion of mulberry leaves to silk. All these findings are in agreement with our results in the late ages. However young instar rearing temperature in our experiments resulted in contrary to Tanaka (1964) who reported that the temperature during young instars (I, II, III) affected the cocoon characters considerably,in which the optimum temperature was 28℃ and the worst was 23℃ concerning the cocoon weight and shell weight. He also recorded the highest shell ratio at 28℃ with the lowest larval loss.

Our results can be explained by the finding of Basavaraju *et al* (1998) who concluded that the temperatur influenced the metabolic activities of the larvae not only depending on age, but also depending on different race. Also it was reported by Singh *et al* (1998) that if the young silkworms are reared under high temperature 27o- 28℃, it is absolutely important to feed them by rich nutritive leaves in sufficient quantity. If the leaf quality and quantity are insufficient, it is necessary to lower the temperature by one degree in each instar, Which means that the infant silkworms, similar to grown silkworms have better utilization of food at lower temperature.

From the data present in Table 3 we can notice that there was not any significant effect of the rearing temperatures concerning the shell ratio. In our opinion this may be explained with the fact that the use secondary and micronutrients gave equal influence on increasing the values of cocoon weight and shell weight characters. However a slight increase was noticed in Experiment 2 with higher temperature rearing which can be explained by the fact that the food digested was utilized the most effectively under the high temperature than in the low and moderate temperatures as reported by Tzenov (1996).

As shown in Fig.1 about the composite result, the cocoon yield per box revealed a significant increase over the control by the low temperature regime 23℃ throughout the whole larval period in

Table3. The economic characteristics of breeds

Character	Exper.	Breed										
		E2	E6	E15	E1	E5a	E14	E4b	E8	E9	E22	E23
Pupation (%)	Exp.1	98.66	98.58	99.04	98.51	98.72	98.73	98.12	98.44	98.58	98.00	98.47
	Exp.2	98.74	98.43	98.23	98.40	98.40	98.40	98.30	98.36	98.45	97.69	98.16
	Exp.3	98.32	98.31	98.25	98.29	98.40	98.22	98.29	98.17	98.40	97.58	98.06
	Contr.	98.74	98.59	98.98	98.84	98.41	98.73	98.62	98.37	98.58	98.03	98.64
Larval Duration	Exp.1	* 705	* 705	* 705	* 681	* 681	* 681	* 705	* 705	* 705	* 705	* 705
	Exp.2	* 657	* 657	* 657	* 633	* 633	* 633	* 657	* 657	* 657	* 657	* 657
	Exp.3	* 657	* 657	* 657	* 633	* 633	* 633	* 657	* 657	* 657	* 657	* 657
	Contr.	681	681	681	657	657	657	681	681	681	681	681
Cocoon Weight (g)	Exp.1	* 2.334	* 2.334	* 2.362	* 2.378	2.462	2.172	* 2.298	2.177	2.093	2.033	2.010
	Exp.2	* 2.106	* 2.063	** 2.002	* 2.097	** 2.001	1.977	* 1.971	1.935	* 1.883	1.827	1.854
	Exp.3	2.183	2.181	2.129	2.195	2.205	2.065	2.081	1.990	1.928	1.871	1.877
	Contr.	2.253	2.199	2.232	2.270	2.380	2.109	2.174	2.078	2.009	1.952	1.919
Cocoon Shell Weight (g)	Exp.1	0.541	0.554	* 0.532	0.507	0.559	0.499	0.503	0.527	0.519	0.442	0.477
	Exp.2	* 0.497	* 0.499	0.448	0.457	* 0.461	0.461	0.441	0.473	0.467	0.405	0.444
	Exp.3	0.509	0.522	0.477	0.471	0.506	0.479	0.462	0.484	0.475	0.414	0.449
	Contr.	0.523	0.525	0.497	0.483	0.544	0.486	0.479	0.503	0.494	0.430	0.458
Shell Percentage (%)	Exp.1	23.16	23.75	22.14	21.33	22.73	22.95	21.87	24.17	24.78	21.72	23.70
	Exp.2	23.43	24.17	22.53	21.76	23.00	23.29	22.35	24.44	24.76	22.16	23.95
	Exp.3	23.31	23.93	22.38	21.47	22.94	23.18	22.2	24.34	24.62	22.13	23.92
	Contr.	23.20	23.86	22.25	21.27	22.87	23.02	22.04	24.21	24.58	22.00	23.87

*P<0.05, **P<0.01, ***P<0.001

Experiment 1, and a significant decrease in high temperature regime 27℃ throughout the whole larval period in Experiment 2, as expected due to the increase and decrease in the cocoon weight respectively without any significant change in the pupation ratio.

However the slight non-significant decrease in the cocoon weight and in pupation ratio manifested itself as a significant decrease in the cocoon yield per box in Experiment 3 where the young instars were reared at 23℃ and late instars at 28℃. Excellence of provided temperature regimes to Experiments 1, 2 and the control were between 2.85-8.75Kg (8.75-23.46%), 2.16-4.87Kg (5.72-12.03%) and 0.50-2.05Kg (1.43-4.92%) respectively. Analogically as cocoon weight character, the highest cocoon yield per box was recorded in breeds with Japanese and Chinese origins.

적 요

수입된 11개 원원 종을 열대기후인 이집트에서 온도가 다른 23℃와 27℃ 에서 사육하여 주요 유전 형질에 미치는 영향을 연구하였다.

저온 및 고온 공히 교잡 종의 화용 비율과 견충 비율에는 영향을 주지 않았으나, 고온은 유충 기간을 단축시켰고, 저온은 견층 중과 상자 당 수견 량을 무겁고 높게 하였다.

따라서 원원 종의 육성과정에서는 유충 전령을 저온으로 사육하는 것이 잡종강세를 극대화 할 수 있음을 알 수 있었다.

DISCUSSIONS

The lower temperature treatment at 23℃ during the whole larval period, instead of the standard regime (27℃ for young and 23℃ for grown larvae) resulted in producing higher cocoon weight, cocoon shell weight and cocoon yield per box by 0.063-0.135gm (2.99-6.14%), 0.012-0.035gm (2.79-7.04%) and 1.24-2.67Kg (2.98-6.14%) respectively.

However, the rearing temperature did not affect the shell ratio and the pupation ratio, thus indicating that this range (23o-27℃) is optimum for silkworm rearing. In general, higher values and proved differences were obtained with breeds of Japanese and Chinese origins while lower ones with genetically sex-limited breeds.

References

1. Ajuzawa 1972. Handbook of silkworm rearing. Fuji pub. Co. Ltd. Tokyo Japan.
2. Basavaraju C.D., Lakshmi Kumari B., Ananthanarayana S.R. 1998. Effect of temperature on consumption and utilization of food in two Breeds of Bombyx mori L., Sericologia 38(4) 615-621.
3. Bashkar R.M., Rajashekar R., Govindappa S. 1999. Effect of temperature on the fertility and gonadal organic composition of silkworm Bombyx mori L. National Conference on Mulberry and Sericulture, Ab No. 151
4. Hanumappa T.V. 1988. Effect of temperature on food and water utilization in different Breeds of the silkworm Bombyx mori L. PhD dissertation Bangalore University Bangalore India 1-91Jolly M.S. 1987. Chawki + rearing concept, organization and management. Indian Silk 25(9), 32-37
5. Jun Liang Xu, Xiao Feng Wu 1992. Research on improvement of efficiency of transforming leaf ingested into the silk of the silkworm Bombyx mori. International Congress of Entomology, Beijing, China Ab. No. 169 003, 23b
6. Karaivanov S. 1989. Studies on the single and complex effect of the temperature, feeding amount and the feeding density on the silkworm Bombyx mori L. development and productivity. PhD thesis Sofia 31pp.
7. Krishnaswamy S.1993. Special features of tropical sericulture and five crucial steps for successful silkworm rearing. Indian Silk, 1, 27-34.
8. Jolly M.S. 1987. Chawki rearing concept, organization and management. Indian Silk 25(9), 32-37.
9. Krishnaswamy S. 1992. The moisture factor in

sericulture, Indian Silk 3,19-21.

10. Lim S.H., Kim Y.T., Lee S.P., Rhee I.J., Lim J.S., Lim B.H. 1990. Sericulture Training manual, FAO, Rome 73pp.
11. Manjula A. 1990. how the cooperative egg production centres in Japan work, Indian Silk, 10, 19-45.Maniraju E., Sekharappa B.M., Raghuraman R. 1999. Effect of temperature on leaf-silk conversion in silkworm Bombyx mori L. Sericologia, 39(2), 225-231.
12. Maniraju E. 1995. Studies on standardization of rearing methods of young age silkworms Bombyx mori L. PhD. Thesis Bangalore Univ. Bang. India, 54-83
13. Matsumura S., Tanaka S., Kosaka T., Suzuki S.1958 Relation of rearing conditions to the ingestion and digestion of mulberry leaves in the silkworm. Sansh, Shikenjo, Hokaku. Tech. Bull. 73:1-40.
14. Maniraju B., Balavenkatsuabbaiah M., Datta R.K.1999. Scientific and practical methods of silkworm disease management, Indian Silk 12,16-20.
15. Petkov M. 1972. Study on the effect of some environmental factors on the silkworm Bombyx mori L. development and productivity PhD Higher Agric. Inst. Plovdiv 44pp
16. Petkov N., Petkov M., Manshev M., Natcheva Y., Alexandrov A. 1989 Handbook of sericulture
17. Rapusas H.R., Gabriel B.P. 1976. Suitable temperature, humidity and larval density in the rearing of Bombyx mori, Philippine Agriculturist, 60(3-4) 130-138.
18. Reali G., Meneghini A. Traevsan M.1990. Bacicoltura Moderna, Edagricole, 162pp
19. Shirota T.1992. Selection of healthy silkworm strain through high temperature rearing of fifth instar larvae. Reports of the Silk Science Research Institute, 40:33-40
20. Sigematsu H., Takeshita H. 1967. On the growth of silk glands and silk proteins production by silkworm reared at various temperatures during the fifth instar, Acta. Sericologia With Japanese origin 65, 125-128.
21. Singh G.P., Mathur V.B., Kamble C.K., Datta R.K. 1998. Young age rearing of silkworm, Bombyx mori L. A review, Sericologia, 38(2) 199-213.
22. Takagashi S., Ueda S.1991. Effect of rearing environment, especially air current upon the body of the silkworm larva Bombyx mori L. in artificial diet rearing With Japanese origin Seri. Sci. With Japanese origin 50(5) 453-456
23. Tanaka Y.1964. Sericology Translated and published by CSB Bombay India pp20-35.
24. Tzenov P.1996. Effect of temperature on the time when the metamorphose hormone of silkworm (Bombyx mori) appears. Bulg. J. Agricultural Science 1,30-31
25. Tzenov P.1996. Effect of temperature during the 5th larval instar on the food dry matter utilization in the silkworm Bombyx mori L., Bulg. J. Agric Sci., 2, 491-495.
26. Tzenov P., Maldenov G.1996. Effect of temperature during the 5th larval instar on the values of the biological and reproductive characters in the silkworm Bombyx mori L. Bulg. J. Agric Sci., 2, 247-257.
27. Ueda S, Marizuka H.1962. Studies on the effect of the rearing temperature affecting the health of the silkworm larvae and upon the

quality of cocoons 1, Effect of temperature in each instar. Acta. Sericologia Jap 41:6-21

28. Ueda S.1963. Studies on the effects of rearing temperature upon the health of the silkworm larvae and upon the amount of cocoon silk production 3, on the effect of lower temperature rearing. J. Seri. Sci. With Japanese origin 33(1) 34-42.
29. Ueda S., Suzuki K.1967. Studies on the growth of the silkworm B. mori l. 1, Chronological changes of the amount of food ingested and digested, body weight and water content and their mutual relationships. Bull. Seri. Exp. Sta. 22(1) 65-67
30. Ueda S., Kumara R., Suzuki K.1969. Study on the growth of Bombyx mori L. 2, Effect of the environment during the rearing period on the larval growth, silk yield and wastes of substances in the reeling. Bull. Seri. Exp. Sta. 23(3) 255-293
31. Ullal S.R., Narisimhanna .N.1987. Handbook of practical sericulture. CSB 126-130 & 213
32. Venugopalpilli S., Krishnaswamy S.1987. Adaptability of silkworm Bombyx mori L. to tropical conditions 3, Studies on the effect of high temperature during later developmental stages of silkworm. Indian J. Seri. 26(2) 63-71.
33. Verma A.N., Atwal A.S.1967. Effect of constant and variable temperature on the development and silk production of Bombyx mori L. (Lepidopteron Bombycid) J. Res. Punjab Agr. Uni. 4, 233-239Yakomoto T., Fujimaki T.1962. Inter strain difference in food efficiency of the silkworm L reared on artificial diet. J. Seri. Sci. With Japanese origin 51, 312-315.
34. Yakomoto T., Fujimaki T.1962. Inter strain difference in food efficiency of the silkworm L reared on artificial diet. J. Seri. Sci. With Japanese origin 51, 312-315.
35. Zlotin A.Z., Bolavin I.P.1988. Information about silkworm.