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DIAPAUSE STORAGE TEMPERATURE INFLUENCE ON SILKWORM LARVA HATCHING RATE

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Abstract

We here try to show if there is a significant difference between hatching rate of two different groups of Bombyx mori eggs, related to humidity, temperature and light. We succeeded to prove that egg preservation temperature is the most important factor that influences the hatching rate.

Key words: silkworms, egg preservation, larva hatching rate

INTRODUCTION

The mulberry silkworm (*Bombyxmori*L.) is very delicate, highly sensitive to environmental fluctuations, and unable to survive extreme natural fluctuation in temperature and humidity because of their long years of domestication since 5000 years. Thus, the adaptability to environmental conditions in the silkworm is quite different from those of wild silkworm and other insects. Temperature, humidity, air circulation, gases, light, and so forth, show a significant interaction in their effect on the physiology of silkworm depending upon the combination of factors and developmental stages affecting growth, development, productivity, and quality of silk (Rahmathulla, 2012).

Unfortunately, Romanian sericulture is now in high regression and, lately, the last company that was in charge with collection and conditioning the silk worm cocoons closed the gates. Therefore, the genetic heritage of Romanian silkworm is in great danger. To prevent the loss of the 72 breeds and hybrids, some actions had begun (Dezmirean et al., 2010). Since 2011, more than 50 breeds and hybrids were maintained alive in USAMV Cluj-Napoca laboratories, in order to enhance the preservation and hatching conditions. The mulberry silkworm develops in a complete metamorphosis, from egg to adult, through larvae and pupae. The egg stage is the most time requesting, for example, the monovoltine breeds case, this takes more than 9 months (Marghitas et al., 2003). To maintain the viability of the eggs, the

embryogenesis time must be reduced using temperature. After the *Bombyx mori* female lays the eggs, the temperature must gradually dropped down to 8 and then to 2.5 °C (Mărghitaș et al., 2003). In the last three years, this study had as objective finding an ideal temperature for egg preservation, but also, to keep a high hatching percentage. This percentage is between 70 and 100%, if the incubation conditions are according the following parameters: 24-26 ˚C, 75-85% humidity and 18 hours illumination period (Mărghitaș et al., 2009).

There is ample literature stating that good quality cocoons are produced within a temperature range of 22–27℃ and that cocoon quality is poorer above these levels (Krishanswami et al., 1973, Datta R.K., 1992).

The effect of low temperature during rearing on some characters of breeding line races were studied (Kremkyremky and Michalska, 1984). Similarly, studies of silkworm breeders (Datta et al., 2001, Pandey and Tripathi, 2008) found that low temperature is always better than higher temperature with reference to productivity of silkworm and larval duration for different instars.

MATERIAL AND METHODS

The necessary data for this study was obtained between years 2012 – 2014, as a result of mulberry silkworm (*Bombyx mori)* rearing period performed in the Faculty of Animal Science and Biotechnology. The main objective is to obtain biological material for research. The materials used in this study are mainly represented by sixty sets of seed originated from thirty univoltine breeds and hybrids of mulberry silkworm. From these sixty sets of eggs used each year in this study, thirty samples were taken from SERICAROM Băneasa and the other thirty samples from the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. These two institutions are the only ones in Romania that make huge efforts to preserve the genetic resource of Romanian sericulture by ensuring optimal environmental conditions during the storage and rearing of mulberry silkworm. In these three years, a total of one hundred and eighty sets of seed were collected.

We here mention that we have used some devices to maintain and monitories the microclimate parameters as the temperature, humidity (Hanna HI 93640 thermo hygrometer) and light during the storage and incubation period.

Regarding the methods we used, first we made a parallel regarding the storage parameters as temperature and humidity, conditions offered by these two institutions each year for a nine months interval. The second step was to ensure the same incubation conditions required for larva hatching in all

these three years. The third step was to determine hatching rate based on the obtained data from the incubation period.

RESULTS AND DISCUTION

The embryogenesis evolution of univoltine mulberry silkworm ***Bombyx mori*** is directly linked with the temperature condition. Being a hundred percent domesticated insect after many years of selection processes, improvement and exploitation, ensuring environmental conditions by humans is mandatory, otherwise this species will not survive in feral conditions. Analyzing the microclimate condition from both storage places we were able to observe that the only difference was given by the storage temperature.

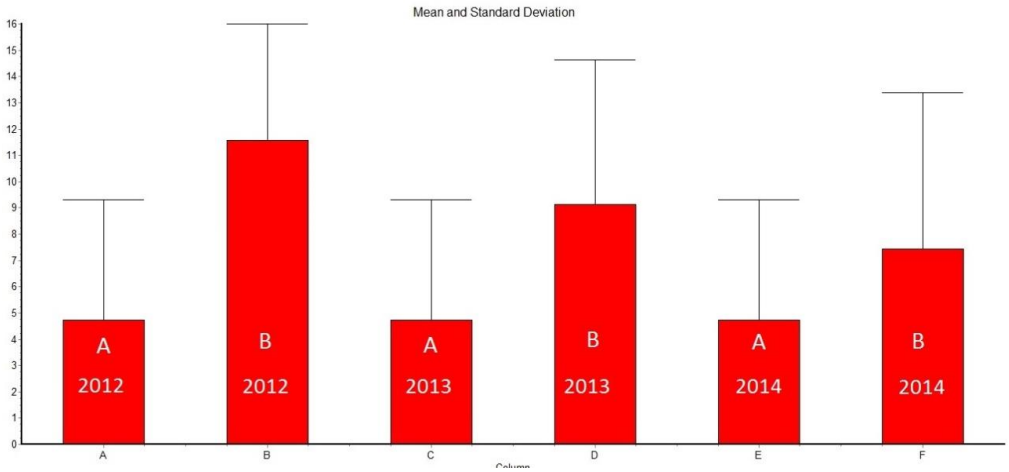

Fig 1. Storage temperature evolution in the last three years in location A and B.
(group A- SERICAROM , group B – USAMV)

Repeated measures analysis of variance applied to temperature variation data shows that the P value is < 0.0001, considered extremely significant for location B data.

Tukey-Kramer multiple comparisons test applied to temperature data obtained from location B explains that every year the temperature conditions were improved in this location, but compared to data from location A, they are significantly different.

Comparison	Mean Difference	q	P value
t USAMV 2012 vs t USAMV 2013	2.429	7.869	*** P<0.001
t USAMV 2012 vs t USAMV 2014	4.143	13.424	*** P<0.001
t USAMV 2013 vs t USAMV 2014	1.714	5.555	** P<0.01

Fig. 2. Comparison of data from group B. (If the value of q is greater than 3.773 then the P value is less than 0.05.)

These differences are caused by the use of different equipment to maintain low temperatures during the storage. It can be seen that in location B where the temperature varies after each season in the last year of the experiment, the temperature was almost equal with the temperature from location A during the storage period.

The main differences that led us to perform this study were those given by the larva hatching percentage from these two locations even if incubation conditions (temperature, humidity and light) were the same.

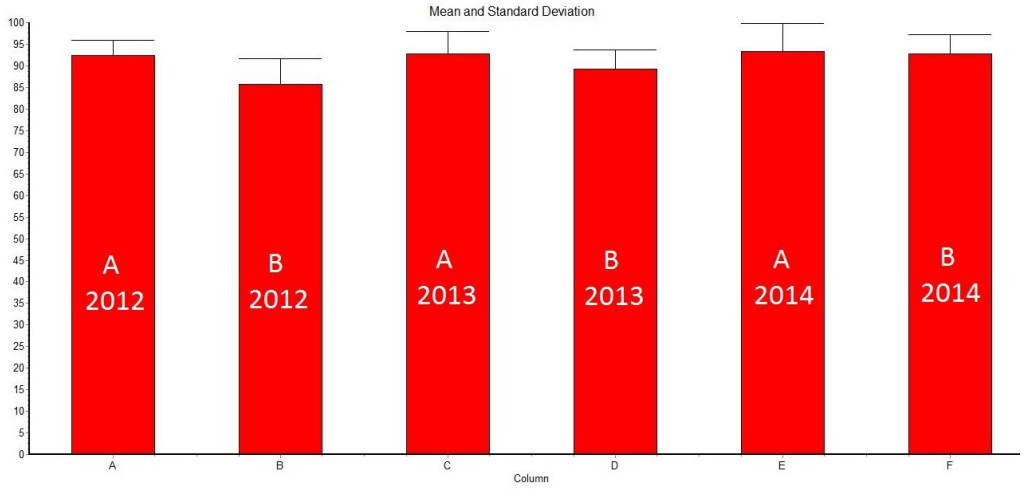

Fig. 3. Hatching rate evolution . (group A- SERICAROM , group B – USAMV)

The Tukey-Kramer multiple comparisons test applied to data from all six years reveals an increase of the larva percentage hatching obtained in group B which almost equals the reference group B performance. If the “q” value is higher than 4.092 then the P value is lower than 0.05.

Comparison	Mean Difference	q		P value
2012 BUC vs 2012 USAMV	6.744	8.161	***	P<0.001
2012 BUC vs 2013 BUC	-0.2837	0.3433	ns	P>0.05
2012 BUC vs 2013 USAMV	3.228	3.906	ns	P>0.05
2012 BUC vs 2014 BUC	-0.9867	1.194	ns	P>0.05
2012 BUC vs 2014 USAMV	-0.3900	0.4719	ns	P>0.05
2012 USAMV vs 2013 BUC	-7.028	8.505	***	P<0.001
2012 USAMV vs 2013 USAMV	-3.516	4.255	*	P<0.05
2012 USAMV vs 2014 BUC	-7.731	9.355	***	P<0.001
2012 USAMV vs 2014 USAMV	-7.134	8.633	***	P<0.001
2013 BUC vs 2013 USAMV	3.512	4.249	*	P<0.05
2013 BUC vs 2014 BUC	-0.7030	0.8507	ns	P>0.05
2013 BUC vs 2014 USAMV	-0.1063	0.1287	ns	P>0.05
2013 USAMV vs 2014 BUC	-4.215	5.100	**	P<0.01
2013 USAMV vs 2014 USAMV	-3.618	4.378	*	P<0.05
2014 BUC vs 2014 USAMV	0.5967	0.7220	ns	P>0.05

Fig.4 Hatching rate comparison between all years and groups

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
2012 BUC	30	92.428	3.590	0.6554	92.830
2012 USAMV	30	85.684	6.065	1.107	86.220
2013 BUC	30	92.712	5.274	0.9628	94.075
2013 USAMV	30	89.200	4.597	0.8392	90.725
2014 BUC	30	93.415	6.442	1.176	95.630
2014 USAMV	30	92.818	4.509	0.8233	94.075

Group	Minimum	Maximum	95% Confidence Interval From	To
2012 BUC	84.040	97.720	91.088	93.769
2012 USAMV	67.170	93.730	83.420	87.948
2013 BUC	74.780	98.900	90.743	94.681
2013 USAMV	76.570	94.250	87.484	90.917
2014 BUC	72.230	98.840	91.010	95.820
2014 USAMV	74.620	97.830	91.135	94.502

Fig. 5 Anova test data summary

CONCLUSIONS

Silkworm breeding aims to achieve superior performances in respect of egg yield, cocoon raw silk yield, cocoon stability and production followed by expansion to new areas besides others. Silkworm breeders continue to strive for an inherent gain in resistance by incorporating resistant genes into the genetic backgrounds of high yielding temperate. Besides this, the cocoon crop stability also relies more on improving other production technologies which have to be explored (Kumar and Singh, 2012). Among the abiotic factors, temperature plays a major role on growth and productivity of silkworm, as it is a poikilothermic (cold blooded) insect (Benchamin and Jolly, 1986).

The data that we have obtained shows us very clear that since in the first two years of experiments the difference of temperature value between the two groups varies significantly, but in the last experimental year, this value arrives at quite close figures. This clearly and directly generates a higher hatching percentage.

Another important factor in optimization of egg preservation is the equipment used in the experiments. Variations of any kind in temperature, humidity and light, directly affects the parameters that we follow.

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