Influence of environmental conditions on the rearing of the silkworm *Bombyx mori* (Lepidoptera: Bombycidae)– Biological consequences of incorrect measurements.

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Abstract

Monitoring and control of environmental conditions are activities of primary importance during the rearing of the silkworm, *Bombyx mori*. Rearing houses must maintain a favorable environment for optimum development of silkworm larvae, and it should not promote the appearance of diseases. The most important environmental factors that influence on silkworm rearing are: temperature, relative humidity, illumination, toxic gas content and air current speed. This review paper discusses about the influence of these environmental factors on the rearing of silkworm, *Bombyx mori*. With it, it is intended to highlight the importance of the correct monitoring of these environmental factors for sericulture industry. Moreover, reference is made to equipment traditionally used for monitoring environmental conditions during silkworm rearing.

Key words: reference values, temperature, relative humidity, illumination, air quality, sericulture

Resumen

El monitoreo y control de las condiciones ambientales son actividades de primordial importancia durante la crianza del gusano de seda, *Bombyx mori*. Los locales de crianza deben mantener un ambiente favorable para el desarrollo óptimo de las larvas, y no debe propiciar la aparición de enfermedades. Los principales factores ambientales que influyen sobre la crianza del gusano de seda son: la temperatura, la humedad relativa, la iluminación, el contenido de gases tóxicos y la ventilación. El presente artículo de revisión bibliográfica discute sobre la influencia de estos factores ambientales sobre la crianza del gusano de seda *Bombyx mori*. Con el mismo, se pretende resaltar la importancia del correcto monitoreo de dichos factores para la industria serícola. Además, se hace referencia a los instrumentos de medición tradicionalmente utilizados para el monitoreo de las condiciones ambientales durante la crianza del gusano de seda.

Palabras clave: valores de referencia, temperatura, humedad relativa, iluminación, calidad del aire, sericultura

Introduction

Metrology means science of measurements. In practical applications, it is the enforcement, verification, and validation of predefined standards. Although metrology is constrained to measurements of length, angles, and other quantities for engineering purposes, that are expressed in linear and angular terms, it is also concerned with industrial inspection and its various techniques. Metrology also deals with establishing units of measurements and their reproduction in the form of standards, ascertaining uniformity of measurements, developing methods of measurement, analyzing the accuracy of methods of measurement, establishing uncertainty of measurement, and investigating causes of measuring errors and subsequently eliminating them[1,2].

Metrology is an indispensable part of the modern-day infrastructure. In fact, it plays an important role in our lives, either directly or indirectly, in various ways. In this competitive world, economic success of most of the industries critically depends on quality and reliability of manufactured products, measurements play a key role in all these requirements [1,3].

Sericulture is the set of cultural and economic activities related to silk. This agri-business has three main components for its success: forestry component, with the cultivation of a perennial plant; livestock component, with the rearing of an insect[4]; and industrial component, with transformation and use of silk thread in textile, cosmetic and medical industry [5].*Bombyx mori* is the most important species used for world silk production. The rearing of this insect dates back more than five millennia, and China was identified as its center of origin.

Bombyx mori is a domesticated insect. The breeding of this insect is carried out under captivity conditions, in facilities prepared for this activity. Growing and physiological parameters of this insect are affected by environmental conditions. Some abiotic factors to control during the rearing of the silkworm are temperature, relative humidity (RH), illumination, toxic gas content (TGC) and air current speed (ACS)[6]. These environmental factors have strictly compliant reference values for successful silkworm rearing.

The aim of this paper is to discuss about the influence of different environmental factors on the rearing of the silkworm *Bombyx mori*. In this way, the importance of correct monitoring of these factors for sericulture industry will be highlighted.

Biological characteristics of silkworm, *Bombyx mori*

Bombyx mori is a silkworm species adapted to commercial rearing. It arose after many years of evolution and artificial selection [7]. It is not found in nature in a free state as it has lost the ability to fly and survive in extreme environmental conditions [8].

Bombyx mori is a complete metamorphosis and monophagous insect, which only feeds on mulberry leaves during the larval stage. In adult stage, this insect only has the mission of perpetuating the species [9]. During embryonic development, fertilized eggs change color. Egg hatching depends on environmental factors such as temperature, humidity [10] and light [11]. Embryo completes its development in a period of 10 d to 12 d before hatching[12] (Figure 1).

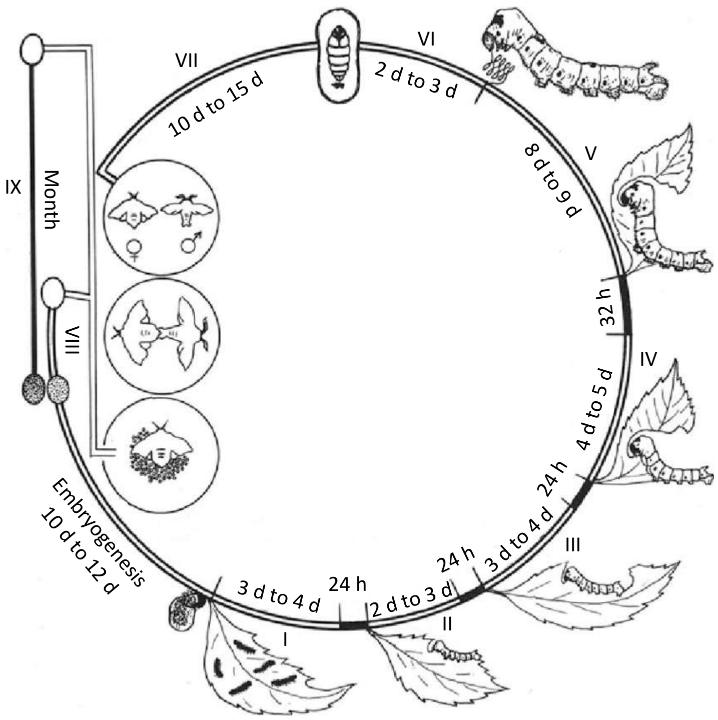

Figure 1. Life cycle of silkworm,*Bombyx mori* (modified from Klimenko,1990 [13]).I-V: Larval instars; VI: Cocoon spinning; VII: Pupal stage. Arabic numbers represent the duration of the instar and the corresponding intermediate molt, in days and hours, respectively; VIII: Artificial hatch; IX: Natural hatch.

Newly hatched larvae are 3 mm long and have the appearance of a hairy caterpillar with a black or dark brown color. During growth, they change color due to development of skin's cuticle. Larvae generally undergo the molting process four times from hatching to cocoon spinning. I instar lasts from 3d to 4 d, II instar lasts from 2d to 3 d, III instar lasts from 3 d to 4 d, IV instar lasts from 4 d to 5 d and V instar lasts from 8 d to 9 d. Whole larval stage lasts 21 d to 25 d [7].

V instar larvae eat 88% of supplied mulberry leaves and they reach their maximum weight one or two days before starting the cocoon spinning. Well-developed larvae weigh about 4 g. Moreover, silk gland is quickly developed during V instar, which occupies up to 40% of its weight. When V instar larvae complete their development and stop eating, the larval integument appears transparent. Silkworm larvae spin cocoons during 2 d or 3 d [4].

At the end of cocoon spinning, silkworms become pupa. Generally, female pupa is bigger than male pupa. After 10 d or 15 d, pupae turn in to moths [14].

Ideal environmental conditions for silkworm rearing

Optimum temperature for normal growth of silkworms is between 20 ºC and 28 ºC, and the desirable temperature for maximum productivity ranges from 24 ºC to 28 ºC. The temperature requirements during early instars are high [12]. At this point, worms feed actively, grow very vigorously, and lead to high growth rate. Such worms can withstand better some adverse conditions in later instars [15].

Early instar silkworms can withstand to high RH than later instar silkworms and under such condition, the growth of silkworms is vigorous. Silkworms can grow without being greatly affected at 90 % of RH or higher, if temperature is maintained at 26 ºC or 28 ºC. Humidity must be lower than 70 % or below to facilitate uniform and good moulting in each instar [6].

Both, temperature and RH, fluctuate widely not only from season to season, but also within the day itself. Therefore, it is necessary to regulate them for the successful crop during the silkworm rearing [8].

Silkworms need high quality air during their development. This air must contain enough oxygen and cannot be polluted. TGC of 1 % CO_2, 1 % formaldehyde gas, 0.02 % SO_2 and 0.1 % NH_3 in the air in rearing house, are optimum limits for rearing silkworms [16]. Early instars larvae are more susceptible to the presence of toxic gas influence. Therefore, air current or stream is needed in rearing houses [9].

Silkworms larvae have optimum development at values from 15 lx to 20 lx of light intensity. Early instar larvae survive better in 8 h light and 16 h dark periods. However, late instar larvae prefer 16 h light and 8 h darkness period. Silkworm larvae do not prefer either strong light or complete darkness but usually light phase, in contrast with dark phase, activates the larvae [6].

Optimum reference values of monitored environmental factors during silkworm rearing are presented in Table 1.

Table 1. Optimum environmental conditions during silkworm rearing during various stages of its life cycle (modified from Rahmathulla, 2012 [6]; and Tzenov [17]).

Environmental factors	I Instar	II Instar	III Instar	IV Instar	V Instar	Spinning
Temperature	28 ºC	27 ºC	26 ºC	25 ºC	24 ºC	25 ºC
RH	From 85% to 90%	85%	From 75% to 80%	From 70% to 75%	From 65% to 70%	70%
Illumination	8L16D			16L8D		
	Light intensity: from 15 lx to 20 lx (or from $0.285\ \mu mol \cdot m^{-2} \cdot s^{-1}$ to $0.380\ \mu mol \cdot m^{-2} \cdot s^{-1}$)					
TGC	[CO_2]=1 %, [SO_2]=0.02 %, [NH_3]=0.1 %, [formaldehyde gas]=1 %					
ACS	1 m/s					

Notes: RH: Relative humidity;8L16D: 8 h light and 16 h dark period; 16L8D: 16 h light and 8 h dark period; TGC: Toxic gas content; ACS: Air current speed.

Temperature

Temperature is probably the most important environmental factor influencing silkworm rearing. *Bombyx mori* is a poikilothermic animal because it is unable to regulate body temperature through internal mechanisms[8,10]. In general terms, early instars (I, II and III) larvae are resistant to high temperature which also helps in improving survival rate and cocoons characters. Temperature has a direct correlation with the growth of silkworms. Increasing or decreasing of temperature by 1 ºC have negative effects on silkworm development. Rise in temperature increases various physiological functions and with a fall in temperature, the physiological activities are decreased. Increased temperatures during silkworm rearing in late instars (IV and V) accelerates larval growth and shortens the larval period. On the other hand, at low temperatures, the larval growth is slowed and the larval period is prolonged [6].

Temperature above 30 ºC directly affects the health of the worm. If temperature is below 20 ºC all the physiological activities are retarded, especially in early instars; as a result, worms become weak and susceptible to various diseases [15,18].Influence of some non-optimal temperature values on silkworm rearing is described in Table 2

Table 2. Influence of non-optimal temperature values on the rearing of silkworm, *Bombyx mori*.

Temperature	Consequences	References
38 ºC	Decreased spinning time	[19]
36 ºC	Decreased cocoon weight	[20,21]
	Larval period is prolonged	[21]
	Poor quality cocoons are produced	[22]
From 35 ºC to 36 ºC	The amount of food consumed by silkworm larvae decreases	[21,23]
35 ºC	Decreased larval weight Decreased silk gland weight	[24]
From 29 ºC to 30 ºC	Decreased pupation rate	[25]
	Pupal period is decreased	[17]
	Decreased pupal weight	
22 ºC	Increased spinning time	[19]
20 ºC	The amount of food consumed by silkworm larvae increases Decreased larval weight Decreased silk gland weight	[21]
Below 20 ºC	All the physiological activities are retarded, especially in early instars	[6]
18 ºC	The amount of food consumed by silkworm larvae decreases	[23]

Relative humidity

RH plays an important role in silkworm rearing. Combined effects of temperature and RH largely determines the satisfactory growth of the silkworms, production of good-quality cocoons and pupation patterns [26].

RH directly influences the physiological functions of the silkworm. It also indirectly influences the rate of withering of the leaves in the silkworms rearing beds. At 55 % of RH, the leaves wither very fast and consumption by larvae will be less [23]. This affects growth of the larvae and results in wastage of leaf in the rearing bed. Retarded growth of young larvae makes them weak and susceptible to diseases

[18]. Influence of some non-optimal humidity values on silkworm rearing is described in Table 3, regardless of temperature.

Table 3. Influence of non-optimal relative humidity values on the rearing of silkworm, *Bombyx mori*, regardless of temperature.

RH	Consequences	References
98 %	Increased spinning time	[19]
95 %	The amount of food consumed by silkworm larvae decreases	[23]
55 %	Decreased pupation rate Increased larval mortality	[25]
From 40 % to 50 %	The amount of food consumed by silkworm larvae decreases	[23]
40 %	Decreased spinning time	[19]
	Decreased cocoon weight Decreased shell weight	[21]

Notes: RH: Relative humidity

Illumination

Silkworms are photosensitive and they have a tendency to crawl towards dim light. They do not like strong light neither complete darkness. Rearing silkworms in continuous light delay their growth. Further, it causes penta-moulters [16]. In bright light there is a tendency towards production of heavy cocoons, and in complete darkness the larval period is shortened. Rearing in complete darkness leads to tri-moulters [27].

Early instars of silkworm larvae show a positive photo-tropism while the late instars show a negative photo-tropism. Therefore the larvae should not be exposed to a direct light [9]. Influence of some photo-periodic regimes and light intensity values on silkworm rearing are described in Table 4.

Table 4. Influence of photo-periodic regimes and light intensity on the rearing of silkworm, *Bombyx mori*.

Stimulus		Consequences	References
Photo-periodic changes inside rearing houses	LL	Increased cocoon weight Increased shell weight Increased pupal weight	[28]
		It leads to M^5	[16]
	DD	It leads to M^3	[27]
		Decreased cocoon weight Decreased shell weight Decreased female pupal weight	[28]
	12L12D	Decreased shell weight in cocoons of male silkworms	
Light intensity changes inside rearing houses	0.1 lx or $0.002\ \mu mol \cdot m^{-2} \cdot s^{-1}$	It induces diapause in few individuals	[29]
	5 lx or $0.095\ \mu mol \cdot m^{-2} \cdot s^{-1}$	It prevents diapause induction	
	Increased light intensity	Larval period is prolonged Pupal period is prolonged Adult period is prolonged Decreased cocoon weight Decreased pupal weight Decreased laid eggs	[30]
	Decreased light intensity	Larval period is shortened Pupal period is shortened Adult period is shortened Increased cocoon weight Increased pupal weight Increased shell weight Increased laid eggs	

Notes: LL: 24 h light; DD: 24 h dark; 12L12D: 12 h light and 12 h dark; M^3: Tri-moulters; M^5: Penta-moulters

Toxic gas content and air current speed

Scientific research on the influence of toxic gas content and air current speed on silkworm rearing are scarce. Silkworm larvae, particularly late instars, have high requirements in oxygen. A bad airing creates conditions that encourage the growth of fungus in bedding and pathogenic agents of the silkworms [14,18]. During silkworm rearing, toxic gases that modify the composition of the air in the facilities are created. Some of these toxic gases are CO, CO_2, NH_3 and SO_2. These gases appear not only by the fermentation of animal and vegetable waste, but also by human activity [6]. Influence of some toxic gas content and air current speed values on silkworm rearing is described in Table 5.

Table 5. Influence of toxic gas content and air current speed on the rearing of silkworm, *Bombyx mori*.

Stimulus		Consequences	References
TGC changes inside rearing houses	$[CO_2]$>1 %	Slowed larval growth	[9]
	$[CO_2]$=10 %	It produces vomiting in larvae	
	$[CO_2]$>10 %	It produces larval death	
	$[NH_3]$: from 0.5 % to 1 %		
ACS changes inside rearing houses	From 0.1 m/s to 0.5 m/s (during V instar)	Reduced larval mortality The amount of food consumed by silkworm larvae increases Increased cocoon weight Decreased spinning time	
	1 m/s (during V instar)		[6]
	1.5 m/s		[4]

Monitoring of environmental conditions

Practice of sericulture industry is traditionally carried out by low-income producers who prioritize increasing production results with the least possible economic expense. The purchase of sophisticated instruments for monitoring the environmental conditions in the breeding premises makes the production process of sericulture industry more expensive for this kind of producers. That is why during silkworm rearing, instruments used to monitor environmental conditions are cheap and depends on their availability in the market.

Temperature monitoring inside rearing houses is usually done with liquid-in-glass thermometers. Regardless their fragility and the lack of remote logging capability, this kind of thermometers do not require an external power supply and can be relatively inexpensive. Moreover, as glass in generally chemically stable, liquid-in-glass thermometers can be used in a wide variety of chemical environments, including lime, chlorine and formaldehyde disinfected environments used for silkworm rearing [31]. Thermometric liquid inside the thin glass container should be mercury, ethanol, toluene or xylene. Also, uncertainty of liquid-in-glass thermometers used during silkworm rearing must not exceed values ranging from ± 1 ºC.

RH monitoring inside rearing houses is usually done with a psychometer, or a wet and dry-bulb thermometer [32]. RH is determined by locating the intersection of the wet and dry-bulb temperatures on a psychometric chart that highlights optimal values of RH during silkworm rearing.

Despite their importance, illumination, TGC and ACS are not monitored inside rearing houses during silkworm rearing. These environmental conditions are monitored only for scientific research. Illumination and ACS of rearing house is established during premises designing process. That is why more sophisticated instruments are used for monitoring these environmental factors inside rearing houses.

Conclusions

Silkworm rearing is strongly influenced by environmental conditions. Small variations in environmental conditions negatively affect rearing performance, cocoon quality and life cycle of *Bombyx mori*. That is why, the use of the appropriate instruments for monitoring of environmental conditions during silkworm rearing, guarantee a large part of the success of sericulture industry. Continuous change of environmental conditions during seasons makes necessary to strictly control environmental conditions in breeding premises.

Recommendation

More studies should be done on the influence of TGC and ACS inside rearing facilities on silkworm rearing.

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