Current Sericulture Situation and the Silkworm Diseases Control in China

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3. Menace of Pebrine Disease in China
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Sericulture (Silkworm+Mulberry+Culture)

• It's an economic, cultural and traditional secondary agricultural activity, which is particularly providing clothes, and additional income to farmers in agro-regions.
1. Introduction

• Sericulture Changes in China

• within 1949 to 2014
  ➢ Recovering (1949-1954)
  ➢ Developing (1955-1959)
  ➢ Adjustment (1960-1964)
  ➢ Developing (1965-1985)
  ➢ Improving and developing (1986-1995)
  ➢ Adjustment(1996-2001)
  ➢ Steady developing (2002-

• Sericulture Current Situation
Sericulture distributed in China

South China 49%
Mid-China 9%
South-West China 17%
North-West China 4%
East China 21%
Tussah 95%

Data adapted from Prof. Xiang ZH
Distribution: Sericulture Reforming

Roll out to other regions (e.g. west of China)

“to improve economy of poor silkworm farmers”
Scale young silkworms co-rearing base （cooperative rearing & saleable young larvae）

Yongfu, Guangxi, West China

Xinda Cocoon and Silk Ltd. Company, Wenyuan, Guangdong
Saving labour, promoting Semi-mechanized Farm Tools etc..

- Autocutting & branch feeding
- Air sterilization
- Mechanization in reeling
The area of mulberry orchards in China
1950-2013

Data from Department of Plantation Management, MOA
The amount of silkworm eggs for distribution and the total output of silkworm cocoons in China

Data from Department of Plantation Management, MOA
The total output value of silkworm cocoons in China

Data from Department of Plantation Management, MOA

The total output value of silkworm cocoons in China

RMB

25 Billion RMB

Year

7/21/2015
Output value per box of eggs and Cocoon production per box

Output value per box of eggs

Cocoon production per box of eggs

Data from Department of Plantation Management, MOA

Figures represent years 1950 to 2012.

Eggs marketing

Crops

R M B kg/b ox

Year

Output value per box of eggs

Cocoon production per box of eggs
The trends of average price of fresh cocoon in China

Data from Department of Plantation Management, MOA

1 EUR = 6.7 RMB

5.97 EUR/kg
Cocoon yield per unit of mulberry land and output value per unit land

Kg/ha

RMB/ha

Data from Department of Plantation Management, MOA

land productivity
Sampling inspection of F1 hybrids in Guangdong (1999-2014)

Data from the Quality Inspection Center of the Ministry of Agriculture
The comprehensive utilization of sericulture resources

mulberry resources
- Mulberry leaves
- Mulberry branches
- Mulberry fruit
- White mulberry root-bark

silkworm resources
- Young silkworms
- Silkworm pupa
- Silkworm moth
- Silkworm excrement
- Natural silk
Wide consensus on efficient development and utilization of sericulture resources has been reached in the industry.
Comprehensive utilization of resources are very rich in sericulture

The national annual output of 600,000 tons of fresh cocoons resources:

- 120,000 tons of silk
- 480,000 tons of fresh pupa
- 1.8 million tons of mulberry branches
- 600,000 tons of mulberry Fruit
- 900,000 tons of silkworm excrement
- 810,000 tons residual mulberry

Sericulture resources all over the country's 26 provinces (cities, districts) more than 1,000 counties
Remarks

✓ **Volume of sericulture production Decreasing but Stable Business Development**
✓ Sericulture swifting to the West-South China
✓ Science & Technology reforming
✓ Land Productivity improvement
✓ Promotion more innovation technology in the fields (Reeling machines, Labors productivity, Marketing )
✓ Diversity in Sericulture.................
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New activities of the occurrence and management of silkworm diseases

virus

pebrine

NPV

bacteria

lack of disinfection
The threat of Viral Diseases

- CPV Disease
- NPV Disease
- CPV&DNV
- NPV Disease
Muscardine & White Muscardine, *Beauveria bassiana* (Bals.) Vuill. high prevalence on June, South China.
Bombyx batryticatus: traditional chinese medicinal materials

White Muscardine, $40USD/kg
New trend of silkworm Pebrine Disease in the field

infected silkworm

uninfected silkworm

pebrined silkworm
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1. Introduction

2. Symptoms of Silkworm Disease

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Symptoms of Pebrine Disease

Pebrine is a devastating parasitic disease caused by microsporidian parasites, mainly *Nosema bombycis* and to a lesser extent *Variomorpha, Pleistophora* and *Thelophania* species.
Pebrine disease in silkworms

- not molting
- Pepper spot
- batch of silkworms
- Infected silk gland
Pebrine disease in China

Over 85% of the sericulture area is infected
Microsporidians discovered from silkworms

I. Different genera:  
   *Vairimorpha* (Pilly, 1976)  
   *Pleisiophora* (Gurley, 1893)  
   *Thelohania* (Henneguy, 1892)  
   *Endoreticulatus* (Wan *et al.*, 1995)  

   **Nosema**

II. Same genera but different species: *Nosema* sp.

III. Same species but different shapes. Sub-stains? serotype?
Microsporidians discovered from silkworms: pebrine & microsporidiosis?

I. Different genera: 

- *Vairimorpha* (Pilly, 1976)
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*Nosema*

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III. Same species but different shapes. Sub-stains? serotype?
Prevalent state of pebrine disease in China

- 1995-2000
- 2000-present

To investigate the epidemic disease in Yingde, and most sericulture area in Guangdong province

![Graph showing the infected ratio of pebrine disease over the years from 1957 to 1997.](image)
Infected pebrine silkworm

Bombyx

cabbage butterfly

diamond back moth

mulberry looper?
*Plutella xylostella* (Diamondback moth)

Microsporidian spores isolated
From *Plutella xylostella*
(abbreviation: XCE)
Antheraea pernyi (China oak silkworm)

N. antheraeae
Phthonandria atrilineata Butler
(Mulberry geometrid)

Microsporidian spores isolated
From Phthonandria atrilineata Butler
(abbreviation: SCH)
Microsporidian spores isolated
From *Pieris rapae* (abbreviation: CFD)
Control pebrine with microscopy
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1. Introduction
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4.1 Influential factors of the occurrence of silkworm diseases and the interrelation

The epidemic factors of silkworm diseases

- abiotic factors
  - Humidity
  - Temperature
  - sunlight
  - rainfall
  - Wind

- biological factors
  - pathogens
  - Mulberry
  - Silkworm

The epidemic factors of silkworm diseases include both abiotic factors such as sunlight, rainfall, Wind, temperature, and humidity, and biological factors such as pathogens and silkworms. These factors interact with each other, affecting the occurrence of silkworm diseases.
How to control the silkworm diseases?

- Pathogens
- Mulberry
- abiotic factors
- Treatment
- Silkworm
4.2 The new ideas of silkworm diseases prevention and controls

How to effectively control of silkworm diseases?
The new ideas of prevention and control the silkworm diseases

- **Diagnosis**
  - Air microorganism detection
  - The silkworm body microbial detection

- **Disinfection**
  - Chlorine dioxide disinfection
  - Ozone disinfection
  - Chemical disinfectants

- **Treatment**
  - Physical treatment methods
  - Chemical treatment methods

**Advantages**
- Easily and quickly
- Environmental safety
- Safe and economic effective
4.3 Prevention and Management of Silkworm Diseases and its practicing

1. Pebrine disease prevention and control system

2. Standard and regulations of disease control

3. Innovation of detection technology of silkworm diseases

4. The reform of disinfection technology

5. Study on the drug for silkworm disease

6. Mulberry pest prevention and control

7. Application of the Internet of things technology
1. Pebrine disease prevention and control system

- Prediction, and control oral infection
- Integrated Pebrine Management
- Systematic Control.
2、Standard and regulations of disease control

- Eggs production
- Young Larvae
- Mulberry fields
3、Innovation of detection technology of silkworm diseases

1）Classified collection of pathogens
2）Separation and identification techniques of *B. bassiana*
3）Development of visual microscope
4）PCR molecular diagnosis
5）LAMP detection protocols
6）Improvement of disinfection technology
7）Exploration of ecological control of the mulberry pests
1）Collection, classification and detection of pathogens
2) Separation and identification techniques of *Beauveria bassiana*

① Separation and purification method of Beauveria bassiana. Patent No. CN201310566645.7

② CN201320717411, Culture device applicable to microorganism separation and purification.
Rapid detection of the environment pathogenic microorganisms

The total number of colonies tests

tests card

Fungal yeast tests card
2）Detection of pesticide residues in mulberry leaf
3) Digital multifunctional microscope

Stereology microscopy & Optical microscopy
The visual microscope research and development
Multi function digital LED microscope to observe the silkworm eggs
Observe the plant diseases and insect pests

plant white powder disease

pest eggs
Observation by light microscope

Nosema bombycis

Microsporidian sp.
Microsporidian sp. stained with Calcofluor White M2R

Observation by fluorescence microscopy

N. bombycis
4) PCR molecular diagnosis technology

**PCR procedures**

- Sample
- Reaction
- Primers
- Buffer
4种微孢子虫全基因组的比较
A rapid DNA extraction method of *Nosema*- Boiling

1: positive control; 2: $10^8$个/mL spores; 3: $10^7$个/mL spores; 4: $10^6$个/mL spores; 5: $10^5$个/mL spores; 6: $10^4$个/mL spores; 7: $10^3$个/mL spores; M: 100bp DNA Ladder.

Fig. *Nosema bombycis* DNA electrophoresis
PCR diagnostic techniques for silkworm pebrine —PCR Diagnostic kit

Fig. Sensitivity of PCR (primers V1f/530r) detection

Left：Diagnostic kit
Right：Several pathogenic microsporidia PCR amplification test results
PCR method for rapid detection of BmDNV

**PCR detection Results**

NB: M. DL2000 DNA 1 ~ 12 oral infected by BmDNV within 1 ~ 12 hrs; 13 ~ 20: 18 ~ 60h (Sample time 6h); 21. BmDNV; 22. silkworm; 23. ddH₂O
RT-PCR method for rapid detection of BmCPV

Result of RT-PCR detection BmCPV

注：M. DL2000 DNA；1. BmCPV；2. silkworm；3. ddH₂O
Detection of *Nosema bombycis* by Loop-mediated Isothermal Amplification

On the basis of separation, purification of *N.bombycis* and collection of microscopy samples of female moths, according to the pseudogene of *N.bombycis*, LAMP primers for detecting species-specific for *N.bombycis* were designed and screened, and the specificity, sensitivity and practicability of production samples also were tested.
Detection of *Nosema bombycis* by Loop-mediated Isothermal Amplification (Idiotype)

Fig. Primers FI2/BI2, F2/B2 LAMP detection

Note: 1: N.b DNA; 2: N.locustae spore DNA; 3: N.a DNA; 4: Normal silkworm midgut DNA; 5: ddH₂O
More

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4. Research and development of disinfection technology

1) Temperature and humidity control and air purification of the production environment

automatic controlling device for the air conditioning equips
2) Evaluation of disinfection effects——
Effective chlorine of disinfectant testing
Hygiene disinfection system for silkworm rearing

Sanitation on Standard Operating Procedure (SSOP)

Disinfection on Standard Operating Procedure (DSOP)
4) Disinfection instrument innovation application

- Air freshener
- Chlorine dioxide (CLO₂) air disinfection machine
- Ozone generator (O₃)
5、Pebrine treatment

- Drug treatment
- Albendazol Tablets
- Carbendazim, Benlate
6. Mulberry pest prevention and control

• Solar insecticidal lamp

*Doaphnia pyloalis* (Walker)
7. To explore with applying of the internet of things (IOT) technology

- Real-time monitoring of silkworm eggs protection
- Silkworm rearing temperature and humidity control
- Rearing environmental air quality monitoring and control
- Real time monitoring of water and fertilizer of mulberry field
- Mulberry pest real-time monitoring
- Real time monitoring of sericulture growth?
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One Belt and One Road
("Silk Road Economic Belt" and the "21st Century Maritime Silk Road")
Cooperation between RSTC and Institutes in Asia-Pacific

1. Jointly implement the sericulture project in your country under the international funds
2. Feasibility report and agreement on cooperation
3. Training for trainers and training for farmers
4. Demonstration farm
5. Supply of silkworm eggs and Mulberry seeds/seedlings to the farmers
6. Technical extension
7. Cocoon and silk processing
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