昭和55年度

インドネシア養蚕開発計画

—病害虫防除—

昭和55年7月

国際協力事業団

はしがき

インドネシア養蚕開発計画は、昭和５１年３月３０日に署名された合意議事録による協力に始まり、昭和５３年２月２８日に締結された「養蚕の分野における技術協力に関する日本国政府とインドネシア共和国政府との間の協定」に基づいて実施されております。

本報告書は、昭和５３年６月２０日から昭和５５年６月１９日までの２年間に亘り、「病害虫防除」専門家として従事された井上元氏の貴重な成果を取りまとめられたものであり、今後の技術協力に大いに活かされることを願うものであります。

末文乍ら、同氏及び同氏の所属先、関係機関に深甚の謝意を表します。

昭和５５年７月

国際協力事業団
農業開発協力部長　金津昭治

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インドネシア養蚕開発計画

― 病害虫防除 ―

派遣専門家
井上　元

私は1978年6月20日から1980年6月19日までの2年間、インドネシア養蚕開発協力計画に病虫害防除の専門家として参画し、カウンターパートの教育・指導ならびに蚕桑病虫害防除の現地適応技術の開発に従事した。この間の業績はつぎの出版物としてとりまとめられ、日イ両国の関係機関に提出された。

1. 報告書：インドネシアの蚕病 ― 現状と防除（英文），51pp. S.D.C.P №13
2. 技術書：蚕桑病虫害防除の研究技術（英文），42pp. S.D.C.P №12
3. 教科書：蚕病防除の指導者（インドネシア語），22pp. S.D.C.P №11

1. 業務の内容と背景

本専門家の担当分野は(1)蚕病の防除、(2)桑害虫の防除、(3)桑病の防除、の現地適応技術の開発にある。カウンターパートは本専門家着任直後に1人（ハサヌディン大学中退）任命され、さらに半年後に1人（ハサヌディン大学卒業）補充された。しかしながら2年の間にカウンターパートは交互に日本研修へ派遣されたので、実際は1名のカウンターパートと2名のアシスタントによって調査または試験が実施された。そのようなわけで、上述の3分野のうちで最も緊急を要する蚕病の防除技術の開発に調査試験の重点を置いた。

当プロジェクトの活動の場である南スラウェシ州における蚕病については1974年の予備調査団の報告のみであったので、まず蚕病の実態を明らかにする目的で諸調査を実施した。その結果に基づいて明らかとなった主要な病気の防除技術の開発のために諸試験を実施した。

2. カウンターパートの教育と指導

カウンターパートの指導は、最初は英語で実施したが、彼等は英語が読むこと以外は不得手であったので、徐々にインドネシア語に切り換え、2年目に入ってからはインドネシア語のみで行なった。調査や試験を実施する際には英文でTextまたは、Experiment planを作成し、事前にそれらをカウンターパートに手渡し、業務の円滑な進行を図った。このTextおよびExperiment planをまとめたものが前述の技術書である。Textはカウンターパートの教育を目的としたもので、主に病気の診断技術の修得に重点を置き、Experiment planは現地適応技術開発の試験の実施方法を主体としている。これらを出版物としてまとめた理由は、

(1)インドネシアには現在蚕桑病虫害防除の技術書が皆無である、(2)技術移転が少数の人に限定されるのを防ぐためである。

現在、蚕業指導員を通じて持ち込まれる農家の病蚕はカウンターパートとアシスタントによって診断されるようになり、病気の診断技術は既に修得したものと言えるだろう。

3. 技術職員と農民の訓練

インドネシア側の推薦する技術職員、とくに蚕業指導員を直接指導する機会は得られなかった。蚕業指導員の研修はカウンターパートが講師となっているが、蚕病防除の適切な教科書がないので、これまでカウンターパートに講義してきたことを、蚕業指導員の研修にふさわしいように内容を配列した教科書をインドネシア語で作成した。これが前述の蚕病防除の指導書である。内容は3章からなっており、防疫の重要性の認識と病気の伝播様式の理解を主眼としたものである。

また、映画「新しい養蚕技術(インドネシア語)」と「新しい蚕病予防システム」をソッペン県のサブセンターとワジョー県、エンレカン県に所在するプロジェクト施設で数回上映し、プロジェクト職員および養蚕農民に対する防疫観念の啓蒙に努めた。

4. インドネシア側への連絡および宣伝

本専門家が業務を実施する場合には前述のTextおよびExperiment planをイ国側へ提出すると共に、調査や試験で得られた知見はNoteとして適宜提出した。Noteは№1～23までで、それらを中心に構成したものが前述の報告書である。その他蚕病の実態やカウンターパートの活動状況をイ国側プロジェクトマネージャーに適宜手紙の形で報告した。

5. 業務の推進

養蚕センターへ引越したのが本専門家の帰国4カ月前であり、業務は主としてサブセンターで実施された。また、本分野の供与機材も大部分は帰国直前に整備されたので、業務は専門家の携行資機材によってなされ、不足資機材はインドネシア側経費または現地業務費で購入した。

また、外部の研究機関、とくにハサヌディン大学と交流を持ち、大学の施設を利用して実験を行なったこともある。

カウンターパートはサブセンターに配置されているので普通は専門家が出張して指導にあたった。2年間を振り返ってみて、指導状況には不十分な点が多々みられるが、2年目に入った頃にはカウンターパートとはお互に気心も知れ、専門家の意図する点を良く理解し、手足のように動いてくれたので、相互のコミュニケーションならびに事業の推進に関しては、全体的に満足のいく状況であった。

6. 蚕病防除の現地適応技術の開発

蚕病防除の技術開発のための調査および試験結果は前述の報告書にとりまとめられている。それらの要点はつぎのとうりである。

(1) 南スラウェシ州における蚕病として、糸状菌病（コオジカビ病、緑きょう病、白きょう病）ウイルス病（NPV，CPV）、微粒子病、細菌病が見い出された。

(2) 死ごもり蛹の死亡原因を調査したところ、コウジカビ病菌とNPV（核多角体病ウイルス）に感染しているものが多かった。コオジカビ病は雨季に目立った。

(3) CPV（細胞質多角体病ウイルス）は、その検出頻度は低いとは言えないが、農家の被害は少ない。

(4) 軟化病ウイルスは検出されなかった。また、幼虫体表の一部が透けてくる病気が見い出された。

(5) 以上のことから、南スラウェシ州における蚕病としては、現在コオジカビ病が最も重大であることが判明した。またNPVも1979年が旱魃の年でもあり、被害が急増した。NPV病は地域型から全州的な流行型にそのパターンが変化したと考えられる。

(6) 過去に猛威をふるった微粒子病は、病原体が農家に残存しているものの、既にその発生量は減少し、もはや糸繭生産に対しては大きな被害を与える心配はないものと考察された。本病が低下した理由は、(a)農家自身による多化性蚕種の採取の禁止、(b)微粒子病検査に合格した日本の F_1 蚕種の利用、(c)当プロジェクトの F_2 蚕種製造における微粒子検査技術の向上、に因るものとみなされる。ただし、現在、ジャワ島や外国（インド）から蚕種が入ってくるが、それらの微粒子検査についての情報はない。もし将来微粒子病が増加することがあれば、これらの要因によるものであろう。

(7) コオジカビ病の防除のための消毒薬剤ならびに消毒方法の開発試験を実施した。日本で一般的に消毒剤として利用されているホルマリンは臭いが強いので、高床式住宅の床下部分でカイコを飼育している農家の消毒には使用困難である。

そこで農家の蚕飼育場所の消毒剤の開発を目的として試験をした結果、次亜塩素酸ソーダー溶液（有効成分 0.04 %）が効果的であった。またこの試薬は蚕体蚕座の消毒にも利用できる目度がついた。そこで5人の蚕業指導員にこの試薬を提供し、農家のカイコ（4－5齢）に毎日1回散布させたところ、いつもは F_2 のカイコは上蔟時（カイコが繭をつくる時）に50%の幼虫が斃死するのだが、それが10～15%に減少したとの報告をうけた。目下試験を継続中である。

(8) サラシ粉、石灰、焼ぬか等現地で安価で簡単に入手できる材料を用いての蚕体蚕座の消毒方法、飼育場所の床（土）の消毒方法を試験中である。

7. 桑害虫防除の現地適応技術の開発

南スラウェシ州における桑害虫としては、コナカイガラムシ、クワノメイガ、クワイトハマキに類似した害虫、カミキリ虫の一種、バッタの一種、カイガラムシ、ハダニ、ゾオムシ、マイマイの一種が見い出された。この中ではコナカイガラムシとスキムシ（クワノメイガ）による被害が大きい。

コナカイガラムシならびにハダニによる被害は乾季の終り頃に顕著である。カイガラムシは洪水をうけた桑園に多い。

コナカイガラムシの被害をうけた桑園の再生のために幼虫の移動状況を観察した。その知見に基づいて、被害桑園の再生のためには、(1)刈りとった枝条をできるだけ早く桑園外へ搬出し焼却すること、(2)その後新芽の出る前に、早い時期に、殺虫剤を散布すること、を指示した。

スキムシの防除として殺虫剤を散布する前に、天敵について調査した。その結果、2種類の寄生蜂が発見され、それらの寄生率は約10％であった。現在殺虫剤による防除方法を試行中である。

8. 桑病防除の現地適応技術の開発

この分野は病気の発見に努めたのみである。その結果、コオヤク病、クワウラウドンコ病、汚葉病が発見され、また一部に、根に基因すると想像される病樹が観察された。被害はいずれも少ない。

9. 検 討 事 項

1980年6月より本分野のカウンターパート2名は養蚕センターへ配置される予定である。今までは養蚕の中心県であるソッペン県のサブセンターにおいて蚕業指導員を通じて持ち込まれる農家の病蚕や諸問題の解決に努力してきた。そのようなわけで、蚕業指導員を通じて農民との接触は良い状態を保っていた。今後はそのような関係が希薄になる心配がある。また、サブセンターでは協定にはないがカウンターパート1名を配置してほしいとの希望がある。イ国側の公式な要請はないが事前に検討する必要があろう。

10. 後任者との引継ぎ

関係各位の尽力によって現地で引継ぎが出来た。この間サブセンターではカウンターパートを含めて業務状況の説明、養蚕農家の現場では問題点の検討がなされた。またインドネシア語の教科書については、より一層現地に適応した内容に改変されることを要望した。

NO. : 12

DEPARTEMEN PERTANIAN
DIREKTORAT JENDERAL KEHUTANAN
PROYEK PEMBINAAN PERSUTERAAN ALAM
SULAWESI SELATAN

RESEARCH TECHNIQUES IN THE CONTROL OF DISEASE AND PEST OF SILKWORM AND MULBERRY.

By

DR. HAJIME INOUE

SERICULTURE DEVELOPMENT
COOPERATION PROJECT
(ATA - 72)
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PREFACE

The author joined the Sericulture Development Cooperation Project in Indonesia as an Expert of Control Pest and Disease of Silkworm and mulberry. During his stay for two years in the district of the South Sulawesi, he engaged in the training of counterparts and in the development of control techniques. This is the Text and Experiment plan used for the purpose mentiond above. The former is used for the training of diagnosis techniq of silkworm diseases, and the latter is mainly forcussed to the survey of silkworm diseases.

The author wishes to express his thanks to Ir. J. Suripto, the manager of this Project and Dr. N. Mori, the leader of Japanese Team, for their encouragement throughout the work.

Bili-Bili, April 1980

H. Inoue

(Dr. Hajime Inoue).

CONTENTS

I. TEXT

1. Purpose of Bioassay Method

Bioassay method is one of the important techniques and is usually used to examine the following matters by the death of test silkworm larvae.

1. Contamination of rearing house with pathogens

When we wish to examine about the contamination of the rearing house with pathogens, first of all we collect the dust of the house. But, the amount of pathogens in the dust is usually quite a few and we cannot directly find the pathogens by a light microscope. Therefore, we inoculate the dust suspension to the larvae by a leaf feeding method and if the larvae died, we determined that there was pathogens in the dust. Then, we disect the dead larvae and observe them by the light microscope, and try to find the true pathogen. Pathogens multiplied very much in these diseased larvae.

2. Find of true pathogen from diseased larvae

When the diseased larvae appears in the rearing tray, usually we disect the larvae and observed the symptoms and then examined the tissues by a light microscope. If we could not find any typical symptoms and pathogens, we homogenize the larvae with sterilized water, smear the homogenate on the mulberry leaf, and observe if the larvae die or not. In the case the larvae died, the presence of pathogens are expected.

3. Virulence of pathogen

When we examine the virulence of pathogens, such as virus, bacteria, pebrine ang fungi, we usually dilute the pathogen with sterilized water as 10-fold or 1:1, 1:10 dilution, and then inoculate the diluted solutions to the test silkworm larvae.

4. Resistance of pathogen to disinfection chemicals

When we wish to examine about the resistance of pathogens to disinfection chemicals, we mix the pathogen with disinfection chemicals, and after the appropriate time, the virulence of pathogens are examined by the leaf feeding method.

2. Detection of Pebrine Spore in Silkworm Egg or Dust of Rearing Room

I. Pebrine inspection of silkworm egg

1. Incubate the silkworm eggs till embryos become a final stage (a stage of body pigmentation stage).
2. Pick up one egg, which is laid at a comparatively late time, on a glass slide.
3. Apply one drop of distilled water onto the sample.
4. Place a cover glass, press and crush the sample.
5. Observe it with a phase contrast or light microscope.

In general, there is pebrine spore in the ist feces of newly hatched larvae and in a dead embryo, when larwae are infected with pebrine through trans ovum transmission.

II. Detection of Pebrine Spore in the dust

When you wish to directly detect pebrine spores from the dust obtained at the silkworm rearing place, the method is that mentioned bellow, but in many cases there observed pebrine spore - like substances, such as spores of fungi ; Therefore, the final confirmation must be done by a bioassay method.

1. Collect dust as much as possible from the rearing place.
2. Add 0,5 % Potassium carbonate solution and then mix it in the mixer for 3 - 5 min.
3. Filtrate it with absobent cotton.
4. Centrifuge it 5 - 10 min at 3,500 rpm or precipitate naturally for 48 hours.
5. Observe the precipitated samples with a phase contrast or light microscope.

3. Wright - Giemsa Staining Method for Pebrine

When the pebrine spore is taken into the larvae through mouth, it germs with the contact of midgut juice. This is called as sporoplasm then it develops to spore through the cause of schizont ---- sporont (after about 54 hour) ---- sporoblast (after about 66 hour) ---- spore (after about 96 hour). Because of the difficulty of seeing this multiple procedure by a light microscope except a phase contrast microscope, the staining method is adopted to observe its multiple form.

Staining procedure

1. Disect the larva and pick up a part of tissue (midgut, fat body)
2. Smear tissue on a glass slide
3. Dry it at room temperature
4. Stain with a wright's solution for 30 sec
5. Add a distilled water on the slide and place it for 2 min
6. Decant the solution
7. Stain with a 40 times dilution of Giemsa's solution for 20 min
8. Decant solution and wash the slide with distilled water briefly
9. Dry it at room temperature
10. Mount and observe.

a) Drop an immersion oil on the stained tissue
Observe it using a 100 x objective lens
After observation, dip the slide into xylene and then after removing the oil, stock it in a box, if necessary.

or

b) Drop a mounting medium and place a cover glass on it
Observe it using 40 x objective lens

Preparing the staining solution

Wright's solution : Use the original concentration
Giemsa's solution : Mix 1 ml of original Giemsa's solution with 39 ml of distilled water.

4. Germination of Pebrine Spore

pebrine spore, when swallowed by silkworm larva, exposes a polar filament and sporoplasm with the action of digestive juice in the midgut. This sporoplasm invades and develops in many tissues. The exposure of polar filament and sporoplasm from the spore is called "germination". The germination of spore is able to be occurred with a chemical treatment.

Method A

1. Drop of a small volume of pebrine spore suspension, which is suspended in a distilled water, on a glass slide.
2. Drop of a small volume of digestive juice of the silkworm larva beside the spore suspension on the same glass slide.
3. Place a cover glass on two drops and immediately observe with a phase contrast microscope.

Method B

1. Prepare a 6 % Hydrogen peroxide (H_2O_2) dilution (Original Hydrogen peroxide solution : 1 ml, Distilled water : 4 ml)
2. Prepare a 0.2 M Potassium chloride (KCl) solution (KCl : 1,5 gram, Distilled water 100 ml)
3. Mix two solutions with a same volume
4. Drop a small volume of the mixture on a glass slide
5. Drop a small volume of pebrine spore suspension beside it on the same slide
6. Place a cover glass on two drops and immediately observe with a phase contrast microscope.

Method C

1. Prepare a 1/20 M Potassium hydroxide (KOH) solution (0.28 g of KOH, 100 ml of distilled water).
2. Mix with a 1/20 M KOH solution and a pebrine spore suspension with a same volume.
3. Leave the mixture for 5 min.
4. Drop a small volume of the mixture on a glass slide.
5. Drop a small volume of body fluid of pupa beside it.
6. Place a cover glass on two drops and immediately observe with a phase contrast microscope.

5. Distinction of Polyhedron and Fat Particle

Although nuclear polyhedron and cytoplasmic polyhedron are similar to fat particle at a glance, the reflection against the light and the shape of polyhedron are not the same with that of fat particle, and it is easier for the person who becomes proficient in the work to distinct them. But, if it is difficult to distinct them, (1) the staining method of fat particle with sudan III or (2) dissolution of polyhedron with sodium hydroxide solution, are recommended.

I. Staining of fat particle with sudan III

Preparing the solution

1. Add 2 g of sudan III into 100 ml of 70 % ethanol
2. keep the solution at room temperature for 1 - 2 days until satulation.

   c.f. In general, ethanol is warmed upto 60 °C, but it is dangerous and not adopted here.
3. Filtrate the solution with a filter paper before use.

Staining

1. Smear the tissue on a glass slide
2. Apply one drop of the solution and place the cover glass on the sample or Place the cover glass on the smeared sample and then add the solution from the edge of cover glass.
3. Fat particle is stained but not polyhedron.

II. Dissolution of polyhedron with sodium hydroxide solution

Preparing the solution

1. Add 2 g of sodium hydroxide (NaOH) into 100 ml of distilled water (about 0.5 M).
2. Keep the solution at room temperature.

Dissolving

1. Smear the tissue on a glass slide and place a cover glass
2. Apply one drop of the solution from the edge of cover glass
3. Polyhedron is dissolved but not fat particle.

6. Discrimination of Nuclear Polyhedron and Cytoplasmic Polyhedron

The multiple site of a cytoplasmic polyhedrosis virus and a nuclear polyhedrosis virus is different such as the former in the mid gut and the latter in many tissue but scarecely in the midgut. This is very useful for determination of disease or discrimination of poly hedra. But as for free polyhedra, the shape and size of polyhedron of two viruses are quite similar and it is difficult to discriminate two types of polyhedron. Then, the staining method has been develop.

Staining method

1. Smear the infected tissue or free polyhedra on a glass slide
2. Dry it at room temperature
3. Fix with anhydrous methanol : pour the methanol on the slide and wait until it vaporize
4. Dip it into boiled water for 5 to 10 sec
5. Dip it into colt water for cooling the slide
6. Stain with a 40 times dilution of Giemsa's solution or 0.1 % eosin, or 1 % bromophenol blue (B.P.B) for 20 min, 2 min and 5 min, respe ctively
7. Mount with a mounting medium
8. Observe the specimen

Staining polyhedra

Staining solution	Nuclear Polyhedron	Cytoplasmic Polyhedron
40 x Giemsa	—	+++
0.1 % eosin	—	+ or ++
1 % B.P.B.	- or +	++ or +++

Grade of stain : +++ ++ + -

Reference

Abe Y. (1973) : Sanshi Kenkyu, 89, 104 - 111.

7. Agar Medium for Cultivation of Fungus

1. Pupa agar medium

Contents :		
	Dried silkworm pupae	100 g
	Sugar	20 g
	Agar	15 g
	Distilled water	1000 ml

1. Dry up silkworm pupae under 100 °C
2. Weigh 100 g of dried pupae and crash it roughly
3. Take it into a 1000 ml - conical beaker and add 500 ml of water
4. Autoclaving it for 20 min
5. After it became cool, filtrate the extracted solution with a filter paper
6. Adjust the volume of fitrated solution to 1000 ml with water
7. Add 20 g of sugar and 15 g of agar into the filtrated solution
8. Heat the solution until agar is melted
9. Take every 10 ml of medium into each test tube
10. Autoclaving for 15 min

2. Rose Bengal agar medium

This medium is used for the separation of Aspergillus sp

Contents :		
	Sodium nitrate ($NaNO_3$)	1 g
	Potassium phosphate, dibasic (K_2HPO_4)	1 g
	Dextrose (Sugar)	10 g
	Rose Bengal	50 - 70 mg
	Agar	15 - 20 g
	Distille Water	1000 ml

1. Weigh chemicals mentioned above
2. Heat the medium in order to melt chemicals, especially agar
3. Take every 10 ml of medium into each test tube
4. Autoclaving for 20 min.

3. Czapek's Sucrose - Nitrate Agar Medium

Contents :		
	Potassium phosphate, dibasic (K_2HPO_4)	1 g
	Potassium chloride (KCL)	0,5 g
	Magnesium sulfate ($MgSO4.\ 7H_2O$)	0,5 g
	Sodium nitrate (NaNO3)	2 g
	Ferrous sulfate	0,01 g
	Saccharose (Sugar)	30 g
	Agar	15 g
	Distilled water	1000 ml

Procedure is the same as No. 2

4. Sabouraud's agar medium

Contents :		
	Peptone	10 g
	Saccharose (Sugar)	40 g
	Agar	15 g
	Distilled water	1000 ml

Procedure is the same as No. 2

5. Potato agar medium

Contents :		
	Potato	200 g
	Sugar	20 g
	Agar	15 - 20 g
	Distilled water	1000 ml

1. Wash potatoes
2. Disinfection of the potato surface with 1000 x HgCl2
3. Peal off potatoes
4. Weigh 200 g of potatoes and cut into small blocks
5. Add 1000 ml of distilled water
6. Boil for 30 min
7. Take supermatant and adjust the volume upto 1000 ml with dis - tilled water
8. Add 20 g of sugar and 15 g of agar
9. Heat until agar is melted
10. Filtrate the medium with absorbent cotton
11. Take every 10 ml of medium into each test tube
12. Autoclaving for 20 min.

8. Detection OF Formalin Resistant Aspergillus sp

In general, Aspergillus sp. is easily able to get resistance to formalin.

Nowadays, there is no observation of such Aspergillus sp. in the South Sulawesi, but in future there may appear resistant strains accompaning with increasing using formalin.

Method .

I. By 0,1 % formalin - Czapek's agar medium

1. Take every 10 ml of Czapek's agar medium into each test tube
2. Add 1 ml of 1 % formalin into half numbers of tubes (Called here as Formalin medium)
3. Test tubes in which formalin is not added are Control medium After mixing, autoclave both test tubes for 15 min at 120°C
4. Incline test tubes till medium becomes solid
5. Cultivate spores of Aspergillus objected with a roop in every 3 test tubes of Formalin medium and Control medium, respectively
6. Keep them at room temperature for 8 - 10 days
7. Compare the growth of Aspergillus spores in both mediums

Aspergillus from	Control medium	Formalin medium
Proje.. Tajuncu	+++ +++ +++	+ + +
Farmer A	+++ +++ +++	- - -

II. By 3 % formalin solution

The method of dipping test of Aspergillus spores is described in the Exp. Plan 6.

9. Quantitative Analysis Of Formaldehyde

Metho.

1. Take 1 g of formalin solution
2. Add distilled water upto 100 ml
3. Take 10 ml of formalin dilution mentioned above
4. Add 50 ml of 0,1 N Iodine Solution
5. Add 20 ml of 1 N KOH (Potassium hydroxide) and then keep it at room temperature for 15 min
6. Add 15 ml of 10 % Sulfuric acid
7. Add 1 ml of Stach solution as an indicator
8. Examine the excess amount of Iodine with dropping of 0,1 N Sodium-thiosulfate solution
9. Use distilled water instead of formalin as control (Blank)
10. Calculate the amount of formaldehyde as
    1 ml of 0,1 N Iodine solution = 1.5015 mg of formaldehyde (HCHO)

For example

Result :

Test	Volume of 0,1 N Sodium thiosulfate Solution (ml)	Average (ml)
Control	49,01	
	49,00	
	49,05	49,05
Formalin	23,90	
	24,10	
	25,05	24,35

Calculation :

$49,05^{ml} - 24,35^{ml} = 24,70^{ml}$

$24,70^{ml} \times 1.5015^{mg} = 37,09^{mg}$ . . . Amount of HCHO in $0,1^{g}$ of water

Therefore, the concentration of formalin is 37 percent.

Preparation of some solutions

1). 0,1 N iodine solution ——— Dissolve 16,5 g of KI (Potassium iodine) With DW (Distilled water) and adjust it upto 100 ml. Add 14 g of Iodine. Add 1 ml of diluted HCL (Hydrochloride solution). Add DW and adjust the solution upto 1000 ml. Keep the 0,1 N iodine solution from the Sun Shine.

Diluted HCL —— Use about 1 N Hydrochloric acid (HCL). Add 1 ml of conc (Original) HCL into 10 ml of D W.

2). 1 N KOH ——— Dissolve 56,11 g of potassium hydroxide with DW and adjust it up to 1000 ml.

3). 0,1 N Sodium thiosulfate ——— Dissolve 24,82 g of sodium thiosulfate with DW and adjust it upto 1000 ml

4). 10 % Sulfate Acid solution —— Take 10 ml of DW and then add 5,7 ml of sulfic acid with caution. After it became cool, adjust it with DW upto 100 ml.

5). Starch solution ——— Mix 1 g of starch (Soluble type) with 10 ml of DW. Pour it into 200 ml of hot distilled water and boil the solution until it becomes semitrasparent. Then take the supernatant.

This solution has to be made on the lest day.

10. Diagnosis of Flacherie Virus Disease with Staining Method of Pyronine-methyl green

A flacherry virus disease, so-called on infectious flacherie, is the most important disease in Japan. This is caused by a virus particle of about 27 u m in diameter, and it is not enclosed with protein coat such as polyhedrone, resulting in a difficult diagnosis. This is very dangerous, because if the virus contamines into the larval tray at their young stage, almost all of larvae dies at the late stage of 5 th instar. Recently, this disease gave a very damage to the sericulture farmers in Brazil. Also, this disease is found in Iran and China. Then, we must survey whether it is exist or not in Indonesia.

At present, the diagnosis methods for a flacherie virus disease have been developed, such as (1) a bioassay method, (2) a pyromine methyl green staining method (Unna-Pappenheim stain), (3) a fluorescent antibody technique, and (4) an immuno agar diffusion. Within these method, No. (3) and No. (4) are more sensitive, but it is difficult to try them here because of shortage of equipments and specipic immune serume. Then, we would like to ditect the disease by a pyronine-methylgreen staining method.

Preparing of staining solution

1. Mix following chemicals

pyronine Y	3 g
methyl green	1,5 g
Distilled water	200 ml

2. Wash the solution with chloroform

Mix chloroform with the same volume of the solution
Shake it and then leave it alone
Collect the solution after the separation of chloroform
Repeat washing 4 to 5 times
this procedure is able to be omitted.

3. This is a stock solution.

4. Make the staining solution as follows :

Stock solution	20 ml
Acetic acid buffer	20 ml
Distilled water	60 ml

The mixture is used for staining.

Preparing of Acetic acid buffer

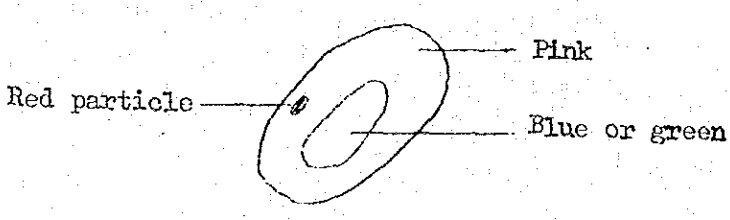
1. 0,1 M Acetic acid solution - Mix of 6 ml of acetic acid with 1000 ml of distilled water.
   0,1 M Sodium acetate solution - Mix of 13,6 g of sodium acetate, cryst, with 100 ml of distilled water.
2. Mix two solution as follows :

0,1 M Acetic acid solution	5 ml
0,1 M Sodium acetate solution	15 ml
Distilled water	20 ml

The mixture is the acetic acid buffer.

Diagnosis prosedure

1. Collect sluggished larvae of 5th instar from farmers
2. Disect them and smear anterior portion of midgut on a glass slide with a pinset or a mess
3. Dry the smeared sample by air for a several minutes
4. Fix the smeared sample with Carnoy's fixative for 1 - 5 min as pouring the fixatives on the sample
5. Wash a glass slide with a distilled water in a moment
6. Pour the staining solution on the smeared sample and keep it for 5 min.
7. Place a cover glass on the smeared sample and observe them
8. If small red particles of 3 - 5 um in diameter in the columnar cell or near the cell as follows, the larvae is determined as to be infected with the flacherie virus



9. Note the reduction of goblet cells

Reference

Iwashita and Kanke (1969) : J. Sericult. Sci. Japan, 38, 64-70.

Preparing of Carnoy's fixative

1. Mix following chemicals

Ethanol (100 %)	60ml
Chloroform	30ml
Acetic acid	10ml

11. Peroral Inoculation of Nuclear Polyhedrosis Virus

According to the research of the dead silkworm cocoon from sericulture farmers, a nuclear polyhedrosis virus (NPV) disease sometimes becomes one of the most important disease. In order to learn the nature of NPV disease, the periode of lethal infection, the median lethal dose, and NPV infection per os are trid.

1. Collection of nuclear polyhedron (NP) from the diseased larva

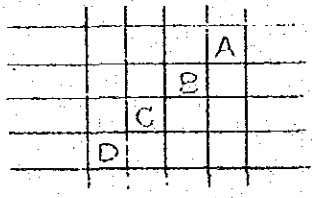
Cut the first pair of prolegs and collect drops of white-colored blood into a test tube. Add a small volume of distilled water. This is the original NP suspension. Usually, NP is purified by the [illegible] centrifugation at a low speed (1,000-3,500 rpm for 10 min) or by the suclose density gradint centrifugation.

2. Decimal dilution of NP suspension

Add 1.8 ml of distilled water into every test tube. Into the 1st tube, add 0.2 ml of original NP suspension. Then, from this mixture, suck up 0.2 ml and add into the 2nd tube. Continue the dilutional work. The original NP suspension is diluted as 10-fold series.

3. Counting polyhedron number

A Thoma hemocytometer is used for counting NP number. As shown in a figure, a 1 mm square is divided into 16 blocks.



Drop a small volume of appropriate dilution. Then, count NP in 4 blocks of them such as A, B, C, D blocks, and calculate a mean number (m). That is,

$\frac{A + B + C + D}{4} = m$ . . . . . mean NP number

$m \times 16$ . . . . . NP number in 0.1 mm^3

$m \times 16 \times 10^4$ . . . . . NP number in 1 ml (10^3 mm^3) of appropriate dilution

$m \times 16 \times 10^4 \times 10^X$ . . . . . NP number in 1 ml of original NP suspension (X =diluted times)

4. NPV infection per os

From the thin NP suspension, NPV is smeared on the both side of mulberry leaf separately with an absorbent cotton. After the leaf is dry, give the leaf to a newly hatched larvae or the 2nd - 5 th instar larvae just after ecdysis. Note the appearance of diseased or dead larva.

5. Calculation of the meadian lethal dose (LD_{50})

See Text 12.

12. Calculation of LD_{50} Value

In general, the virulency of pathogen is revealed by a LD_{50} value (meadian lethal dose) and the effectiveness of disinfective chemicals to pathogen is compaired with the change of LD_{50} value. Thus, the caluculation of LD_{50} one of the fundamental techniques in the insect pathology. Because of the most popular in the insect pathology, the Reed and Muench method (1938) is adopted here.

Example : The result of NPV inoculation

Number of polyhedron	No. of larvae tested	No. of live larvae	No. Of dead larvae	Cumulative No. of Live larvae	Cumulative No. of Dead larvae	Cumulative mortality (%)
10^7	10	0	10	0	22	100
10^6	10	2	8	2	12	86
10^5	10	6	4	8	4	33
10^4	10	10	0	18	0	0

The larvae tested died as presented in the table. Get the cumulative number of live or dead larvae by adding numbers of live or dead larvae from the above or bottom as illustrated by arrows. Get the cumulati mortality is the dose between 10^6 and 10^5

Calculation

$$1.\ LD_{50} = 6 - \frac{86-50}{86-33}$$

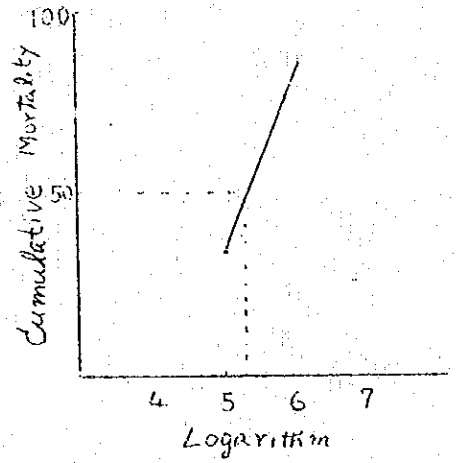
$$= 6 - 0.67$$

$$= 5.33$$

$$LD_{50} = 10^{5,3}$$

$$\therefore \log LD_{50} = 5,3$$

2. Graphic method



Reference

Reed, L.J. and Muench H. (1938) : A simple method of estimating fifty peroent endpoints, The Am. J. of Hygine, 27, 493 - 497.

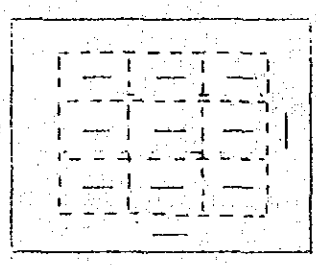
13. Sampling of Pest Insect

Sampling of pest insect is planned standing on its purpose, kind of insect, condition of mulberry field and cultivated method of mulberry.

Herewith, sampling plan is focused to mulberry pyralid, mulberry mealy bug and mite.

For example

1. Divide a mulberry field as shown in a figure and select 3 - 5 trees from each block.



2. Selection of shoots for the survey as follows :

Pest insect	Unit	Selection of Unit
Mulberry pyralid	Shoot	3 Shoots from each tree
Mulberry mealy bug	Shoot	3 Shoots from each tree
Mite	Leaf	5-10 leaves from each tree

3. Counting of the pest number is performed at upper and middle parts of the shoot.
   Count the number of mulberry pyralid or mulberry mealy bug on each shoot.
   Count the number of mite on each leaf.

4. Treat the data statistically (See Text 14).

14. Estimation of Population Density of Pest Insect

A rapid graphic method for the estimation of population density of pest insect is introduced.

For Example

1. The number of mulberry pyralid is counted as follows :
   (1) Every 10 trees is selected at 8 parts (A-H) of a mulberry field
   (2) Every 5 shoots is selected from each tree
2. That is, total shoots observed are 10 x 5 = 50 in every part.
   The result is as follows :

No. of larvae on one shoot	No. of shoots at every part of field								Total shoots
	A	B	C	D	E	F	G	H	
0	46	40	43	38	41	37	44	42	331
1	3	2	7	8	8	5	2	5	40
2	1	3	0	2	1	2	3	2	14
3	0	2	0	0	0	3	0	0	5
4	0	2	0	0	0	2	1	0	5
5	0	0	0	2	0	0	0	0	2
6	0	1	0	0	0	1	0	1	3
Total shoots observed	50	50	50	50	50	50	50	50	400

3. The frequency of the larval number is very many at 0 (zero) and 1 (one), and the A_1 (0,1) graph from shiomi (1978) is adopted.
4. That is,
   (1) Total shoot number (N) observed is 400 shoots.
   (2) Total shoot number of 0 larva is 331 shoots.
   (3) Total shoot number of 1 larva is 40 shoots.
   From this, rerative frequency P_2 is 40/400 = 0.1000
5. P_1 and P_2 are introduced into the A_1 (0,1) graph of Shiomi (1978)

6. Then, the estimated density ($\hat{u}$) and the precision (S) are obtained, That is,

$$\hat{u} = 0.35$$

$$S = 2$$

From this value the large sample variance times ($V_{\hat{u}}$) is obtainable, that is,

$$V_{\hat{u}} = S/N = 2/400 = 0.005.$$

7. The 95 % confidence limits (u) is calculated. That is,

$$u = \hat{u} \pm 1.96 \sqrt{V_{\hat{u}}}$$

$$= 0.35 \pm 1.96 \sqrt{0.005}$$

$$= 0.35 \pm 0.137$$

8. From the calculation mentioned above,
   (1) The average number of larvae in one shoot is 0.35 larvae
   (2) The number of larva in one shoot is 0.2 - 0.5 larvae, because the 95 % confidence limits is 0.213 - 0.487 larvae
9. If every mulberry tree has 10 shoots,
   (1) There are 2 - 5 larvae in one tree

Reference

Shiomi M. (1978) : A rapid graphic estimation of population density by a quasi - sequential method, Bull. Natl. Inst. Agric. Sci. Ser. A25, 33 - 57.

II. EXPERIMENT PLAN

1. Research on The Pathogenical Contamination and Effect of Disinfection at Soppeng Subcenter

At present, diseased larvae appeared at the silkworm rearing - rooms of Soppeng Subcenter. For diminishing this situation, we are going to perform disinfection of the rooms with formaldehyde. At the same time, we examine the pathogenical contamination, if any, and the effective of disinfection.

Material & Method

1. Collect dust from rearing rooms, mulberry stock room, mounting - room, and pebrine examine room before and after disinfection.
2. Add a small volume of distilled, steralized water to the dust.
3. Smear the dust suspension on a both side of mulberry leaf.
4. Give it to the newly hatched larvae for 1 to 3 days after the leaf was dried.
5. Then, give a normal fresh leaf to the larvae every day, and rear them till 4th instar.
6. Check the number of the diseased larvae.
7. Examine the diseased larvae with microscope.

Note

1. When you collect the dust, you should change the collecting tools every gathering place.
2. Please wash your hand well before and after collecting the dust.
3.

Place	Dust before disinfection	Dust after 1st disinfection	Dust after 2nd disinfection
Rearing room A			
" " B			
" " C			
Mulberry stock room			
Mounting room			
Pebrine inspection room			

Control larvae are reared only with normal fresh leaves.

4. When you rear the test larvae, feeding tools should be changed every lot.

Property No. :

Page :

?	No. of test larvae	No. of diseased larvae — Days after inoculation					. . . . .				
		1	2	3	4	5	. . . . .	18	19	20	21
Silkworm strain											
Bioassay memo											

2. Survey on The Silkworm Disease

In the South Sulawesi, it is separated into a wet season and a dry season.

Although the rearing of silkworm larvae in the South Sulawesi is performed through a year, the conditions for silkworm rearing is seemed to be different in these two seasons. The appearance of silkworm disease is also seemed to be different. This survey is focused to research the silkworm disease in the wet and dry season.

I. Disease in the larval stage

Collect the sluggished larvae from the Projects, Units and farmers, and examine the kind of disease.

Place	Larval stage	F1 or F2	No. of larvae examined	Kind of disease					Remarks
				NPV	CPV	Pebrine	Fungus	Bcteria Others	

II. Dead cocoan

Collect cocoons of 50 - 100 at random from the Projects and farmers, and then the [illegible] examine the kind of disease.

Place	No. of cocoon examined	No. of healthy cocoon	No. of dead cocoon	Mortality percent	Kind of disease				Remarks
					NPV	CPV	Pebrine	Fungus Bacteria Others	

3. Distribution of Pebrine Spore

Pebrine disease was the most important silkworm disease in the South Sulawesi, but it decreased gradually owing to the use of hybrid silkworm race and improvement of pebrine inspection etc. However, larvae infected with pebrine are sometimes observed at farmer's houses and we wish to know the distribution of pebrine spore at the Project equipment, Unit and farmer's house.

I. Bioassay

1. Collect the dust from the Project equipment, Unit and farmer's house.
2. Add a small volume of sterilized water and mix them.
3. Smear the mixture on the [illegible] of mulberry [illegible] with absorbent cotton.
4. Give the leaf to the newly hatched larvae for 1-3 days after the leaf became dry.
5. Adjust the number of the larvae as 10-20 every sample next day and rear them in the petri dish.
6. Disect and examine the diseased larvae.
7. Examine all the larvae including healthy one at the end of examination.
8. Examine the diseased larvae about the existence of other pathogens as well as pebrine.
9. Result

Name of place	No. of test larvae	No. of healthy larvae	No. of diseased larvae	Result of the observation				
				Pebrine	NPV	CPV	Fungus	Others
Unit A								
Farmer1								
2								
3								
Unit B								
Farmer1								
2								
3								

II. Obsevation of slaggished larvae obtained at the unit and farmer's house.

1. Collect the slaggished and under - grown larvae.

2. Disect and examine the pathogens as well as pebrine spore.

3. Result

Name of place	Larval instar	No. of larvae examined	Result of examination				
			Pebrine	NPV	CPV	Fungus	Others

4. Detection of *Aspergillus* sp. by Stamp-Agar Method

The results of survey on the dead cocoons in January and March suggested that the main cause of the death of larvae or pupae was Aspergillus disease.

This time, we wish to survey the distribution of *Aspergillus* sp. in the rearing equipments of the silkworm larvae using the stamp-agar method.

Implements

1. Stamp-agar ---- Rose bengal agar plate packed into a vinyl tube and is selled by "Eiken kagaku K.K.", Japan.
2. Absorbent cotton in 70 % ethanol.
3. Petri dish.
4. Knife.

Method

1. Wipe the surface of Stamp-agar (SA) and a knife with 70 % ethanol absorbent cotton.
2. Cut SA as 0.5-1 cm thick from the bottom. This is not use.
3. Push SA and cut it as 0.5 cm thick. This is the control for the check of the contamination of knife.
4. Push SA and touch the research object.
5. Cut it as 0.5 cm thick and place into the disinfected petri dish.
6. Incubate for 2 days at room temperature and examine the colony of *Aspergillus* sp.

Attention

1. The knife must be wiped with 70 % ethanol-absorbent cotton in every use.
2. The petri dish must be disinfected at 160°C for 1 hour under the dry condition before use.
3. The used agar plate must be thrown away after disinfection.

Survey

Place	Rearing room			Mulberry stockroom	Rearing tray
	Floor	Wall	Ceiling		

5. Detection of Flacherie Virus Disease

flacherie virus possesses very strong infectivity and if the disease caused by this virus spread in this country, sericulture - famers will be faced to serious damage. From this view point, we - perform its survey.

Method

1. Collect sluggished 5 th instar larvae of 5 - 7 days old from - farmers.
2. Detect the anterior portion of midgut by the pyronine - Methyl green staing method as shown in Text 10
3. Continue the detection work through a year.

Note

1. Observe 5 - 20 larvae in one farmers as follows :

Place		No. of larvae observed	Flacherie Virus +	-
Unit	A			
Farmer	A			
"	B			
"	C			

2. Examine emphatically, if almost all of larvae dies at the late stage of 5 th instar in some farmers.

Reference

See Text 10 — Diagnosis of a flacherie virus disease with a - staining method of pyronine - methyl green.

6. Inactivation Test of *Aspergillus* sp

According to our survey, *Aspergillus* disease is one of the most important silkworm disease in the South Sulawesi. For the development of preventive techniques of the disease, we will examine the disinfective effect of several chemicals.

METHOD

Preparation of medium

1. Prepare the following medium :

Czapek's agar medium			Czapek's solution		
K_2HPO_4	1	g	K_2HPO_4	1	g
KCl	0.5	g	KCl	0.5	g
$MgSO_4 \cdot 7H_2O$	0.5	g	$MgSO_4 \cdot 7H_2O$	0.5	g
$NaNO_3$	2	g	$NaNO_3$	2	g
$FeSO_4 \cdot 7H_2O$	0.01	g	$FeSO_4 \cdot 7H_2O$	0.01	g
Sucrose	30	g	Sucrose	30	g
Agar	15	g	Distilled water	1000	ml
Distilled water	1000	ml			

Heat and dissolve them.

2. Divide a 10 ml of the medium into every test tube
3. Autoclaving at 120°C for 15 min
4. Pour a 10 ml of Czapek's agar medium from a test tube into a petri dish, which was disinfected at 160°C for 30 min in advance.
   Incline the rest tubes of Czapek's agar medium.
   Keep test tubes of Czapek's solution vertically.
5. Prepare the sterilized distilled water by autoclaving
   a) 10 ml of distilled water in a test tube.
   b) 100 ml of distilled water (DW) with 0.05 ml of Tween 40 in a conical beaker, and then divide 10 ml of them into every test tube.

Cultivation of Aspergillus sp.

1. Cultivate Aspergillus sp. on a Czapek's agar plate
   a) Separate Aspergillus sp. from a silkworm rearing room by the stamp agar method in advance.
   b) Susped Aspergillus spores in the distilled water contains tween 40.

      Spore 1 loop + DW 1ml ---------- suspension 1 : 1
      1 " + " 10 ---------- " 1 : 10

   c) Smear 1 loop volume of spore suspension on a Czapek's agar plate in a test tube or a petridish.
   d) Cultivate them for 3 to 10 days at room temperature.
   e) Then, suspend spores as 1 : 1 suspension.
   f) Cultivate them for 3 to 10 days at room temperature (pure - cultivation).

Inactivation test

1. Prepare the following dilution of chemicals
   Formalin : 3 % (formalin 37 % 10 ml + DW 110 ml)
   5 % (formalin 37 % 15 ml + DW 95 ml)
   Sodium hypochlorite : 0.4 %, 0.04 %, 0.004 % (sodium hypochlorite of 4 % effective con.
   10 ml + DW 90 ml —— this is 0.4 % dilution, then dilute as 10 fold series).
   Calcium hypochlorite : 200 x (0.5 %) (calcium hypochlorite 1g + DW 199 ml).
   500 x (0.2 %) (calcium hypochlorite) 1g + DW 499 ml).
2. Prepare a 1 : 1 spore suspension with distilled water contains Tween 40.
3. Prepare pieces of filter paper No. 2) of about 0.6 cm in diameter and heat them at 160°C for 30 min.
4. Place about 10 pieces of filter paper in a petri dish (9 cm)
5. Drop 1 loop volume of spore suspension on each filter paper
6. Dry them for 1 - 3 hr at room temperature.

7. Add about 20 ml of a disinfective chemicals in the petridish, and after the suitable time of test, transfer the paper into a test with Czapek's solution.

   Ex. Solution 3 ml ——— 1 piece

   " 10 ml ——— 2 piece

8. Cultivate for 7 - 10 days at room temperature

9. Observe the growth of Aspergillus sp.

Result

Chemicals	No.	Dipping time (hr) 0.5	1	3	5
Control	A				
	B				
	C				
Formalin 3 %	A				
	B				
	C				
5 %	A				
	B				
	C				
Sodium hypochlorite 0.4 %	A				
	B				
	C				
0.04 %	A				
	B				
	C				
0.004 %	A				
	B				
	C				
Calcium hypochlorite 200x	A				
	B				
	C				
500x	A				
	B				
	C				

7. Spray Test of Sodium Hypochlorite Solution to *Aspergillus* sp. in The Silkworm Rearing Room

We are thinking to use sodium hypochlorite solution as an disinfectant at the farmer's level, and we already know its disinfective effect to *Aspergillus* Spore in a test tube. This time, we intend to examine its disinfective effect to the spore at the silkworm rearing room.

Method

1. Detection of *Aspergillus* sp. in silkworm rearing rooms by a stamp agar method.
2. Cultivate them for 3 days and know the existence of *Aspergillus* sp in the rearing rooms.
3. Spray sodium hypochlorite solution of 0.004 % and 0.04 in the rooms, respectively, such as,
   Rearing room A . . . . 0.004 % of sodium hypochlorite ($1^{l}/m^{2}$)
   Rearing room B . . . . 0.04 % " " ($1^{l}/m^{2}$)
   Rearing room C . . . . 3 % of formalin as a control ($3^{l}/3.3\ m^{2}$)
4. Close the rooms after the spray
5. On the next day, examine again the existence of *Aspergillus* sp. in the rooms by the stamp agar method.

Result

1. Sodium hypochlorite solution of 0.004 %

	Ceiling	wall	Floor	Raring tray
Before disinfection				
After disinfection				

2. Sodium hypochlorite solution of 0.04 %
3. Formalin of 3 %.

8. Examination on The Utilization of Sodium Hypochlorite Solution as A Disinfectant of Larval Body Surface and Rearing Bed.

The disinfection of silkworm body surface and rearing bed is very important as well as the disinfection of rearing room and tools in the tropical sericulture. Herewith, we examine the possibility of utilization of sodium hypochlorite solution (S.H) for the purpose mentioned above.

Silkworm rearing and examination

1. The method of silkworm rearing is the same as that performed at the Subcentre, Soppeng.
2. The number of silkworm larvae in every test group is more than 1500 larvae at the time of " Hakitate ".
3. At Hakitate, count the number of larvae used.
4. Examine the growth of larvae in every test group and note the S.H. spray times.
5. At the Ist day of 4th instar, count the number of larvae in every test groop and then calculate " The percentage decrease in silkworm number ".

$$\frac{\text{No. of 4th instar larvae}}{\text{No. of Larvae at Hakitate}} \times 100 =$$

6. Then, arrange the number of larvae as 200 larvae in each test described in the Test.
7. At the mounting time of the larvae, count the number of matured larvae.
8. At the time of " Komo-muki (taking away of the mat) ", count the number of dead larvae in the cocooning frame.
9. Harvest cocoons.
10. Count the number of thin and bad cocoons.
11. Count the number of healthy and dead pupae; calculate the survival rate of pupae.
12. Examine the coocon weight, cocoon shell weight of healthy male and female pupae, respectively.

Examination

1. Effect of S.H. sprayed in the rearing room to the larval growth (connected with Exp. plan 7) ; compare the growth of the larvae in Test I and Test IV.
2. The percentage decrease in silkworm number at the Ist day of 4th instar.
3. The survival rate of pupae.

Test no.	No. of 4 th instar larvae tested	No. of dead larvae in 4th-5th instar	No. of dead larvae in cocooning frame	No. of bad Cocoon	No. of dead pupae	No. of healthy pupae	Survival rate of pupae (%)
I-1-A	200	1	2	10	30	157	78,5

4. Cocoan weight, cocoon shell weight and cocoon shell percentage

Test no.	No. of healthy pupae			Cocoon weight			Cocoon shell weight			Cocoon shell percentage		
		0	0	0	0	total	0	0	total	0	0	total
I-1-A	157	80	77	-	-	-	-	-	-	-	-	-

Test

1. See the following paper
2. Tests are duplicated

Test No.	1 – 3 Instar	4 – 5 Instar	Mounting time
I	Papsol every instar (after ecdysis)	1. Papsol every instar + S.H. 0,0 4 % every day	S.H. 0,04 %
		2. Papsol every instar + S.H. 0,04 % every other day	S.H. 0,04 %
		3. Papsol every instar	
		4. Kapur at 5th instar	
II	Papsol every instar + S.H. 0,04% every day	1. Papsol every instar + S.H. 0,04 % every day	S.H. 0,04 %
		2. Papsol every instar	
III	Papsol every instar (Formalin spraid room)	1. Papsol every instar	

9. Test on The Effect of Disinfectants to Aspergillus Spore on The Surface of Silkworm Larva

In order to develop the disinfection technigue of the larval - survace, We wish to do examinations mentioned below :

I. Test of the effect of disinfectants sold in Japan to the Aspergillus sp. obtained in the South Sulawesi

II. Test of disinfection effect of a chemical (Soda + Kapur) to the Aspergillus Spore.

Method

1. Cultivate Aspergillus sp. at an agar medium for 4 - 10 days.
2. Prepare the spore concentration mentioned below.
3. Inoculate spores on the larval surface with the dipping method
4. Keep the larvae inoculated for 1 - 3 hours till the surface is dry.
5. Spray a disinfectant on the larval surface mentioned below.
6. Give mulberry leaves after 30 min - 1 hour.
7. Close plastic boxes for 24 hours.
8. Observe daily the number of dead larvae as below.
9.

Note	Larval instar				
	1 st	2 nd	3 rd	4 th	5 th
Spore concentration	1 : 100 1 : 500	1 : 50 1 : 500	1 : 50 1 : 500	1 : 10 1 : 100	1 : 1 1:10
Disinfectant volume Per M^2	10 g	20 g	30 g	40 g	50 g
Observation	Till 3 rd instar	4 th instar	5 th instar		Matured larvae
	Or For	10 - 12 days			

Test	Disinfectant	No. of dead larvae
I	Pafsol	
	Kabinoran	
	Kemikuron	
	New dast	
	Aspergillus only	
	Control	
II	Soda 5 g + kapur 95 g	
	Soda 10 g + kapur 90 g	
	Soda 15 g + kapur 85 g	
	Soda 20 g + kapur 80 g	
	Aspergillus only	
	Control	

The test is performed as duplicate.

10. Survey on The Natural Enemy of Pest Insect

The insecticide was sprayed in large quantities for the control of pest insect in the Europe, America and Japan in the past year, and it produced the appearance of resistant strain of pest, polution and so on, and nowdays the integrated pest control using such as natural enemies and sterilized insect are thought. The pest control in the South Sulawesi would be mainly performed with insecticides, and we wish to know the actual condition of natural enemies to the main - mulberry pests in advance.

I. Survey on the parastic wasp of the mulberry pyralid

1. Collect 50-100 larvae of mulberry pyralid from the mulberry field.
2. Rear the larvae one by one in the petri dish after note their instar or lenght.
3. Examine the appearance of the parasitic wasp from larva or pupa
4. If the larvae died during the rearing, examine the existence of pathogens.
5. The record of rearing of the larvae

Larval No.	Instar or Body length	Days after the collect 1 2 3 ~~~~ 10	Appearance of Parasitic wasp
1			
2			
.			
.			

6. Result

No. of larvae examined	No. of healthy larvae	No. of dead larvae	Mortality	Appearance rate of parasitic wasp	Existence of pathogens

II. Survey on the mortality of mulberry pyralid at the mulberry field

1. Examine the rate of the dead larvae in the mulberry field at which insecticide is not sprayed.
2. Collect the dead larvae and disect them in the laboratry in order to know the existence of pathogens.
3. Result

No. of larvae examined	No. of healthy larvae	No. of dead larvae	Mortality	Existence of pathogens	Note on the cause of the death

11. Survey on The Transmigration of Mulberry Mealy Bug after Cutting of Mulberry Shoot

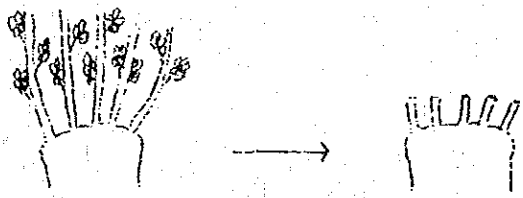
The mulberry mealy bug (Pseudococcus comstocki Kuwana ; Maconellicoccus hirsutus Green) is the worst pest in the South Sulawesi and it gives a marked damage especially in the dry season. The afflicted bud is curled and is not able to grow with the procedure as described in the " Textbook of tropical sericulture ". The insect pest in the curled leaves is usually difficult to be killed by an insecticide. In addition, the spraying time of insectiside is restricted by the time of silkworm rearing.

Thus, the protection of mulberry bud from the mealy bug after the cutting of mulberry shoots is seemed to be the most important for the diminishing of damage.

This survey is performed on the transmigration of mealy bug to the mulberry stump after cutting of its shoots in order to determine the most effective time of spraying insecticide.

Method

1. Select a mulberry field showing a marked damage by mealy bug. Take a photograph of damaged condition of the tree in the mulberry field.
2. Cut their shoots as shown in a figure.



3. Choice 3 - 10 mulberry stumps in Field A and B.
   - Field A - in the case that the cut shoots were immediately brought out of the mulberry field.
   - Field B - in the case that the cut shoots were kept in the mulberry field.
4. Pick up 1 - 3 shoots from every mulberry stump of Field A and B.

5. Count the number of mealy bug on day 1 and 3 after shoot-cutting, if possible and/or take photographs of transmigration of mealy bug.

6. Observe the growth of mulberry bud in the field as follows :

Field A - 1 : Spraying insecticide before budding

2 : disspraying insecticide

Field B - 1 : Spraying insecticide before budding

2 : disspraying insecticide

Count the number of galls in 1 - 3 each mulberry stumps and/or take photographs of growth phase of the bud.

Other

1. Discuss on the suitable spraying time of insecticide

2. Try to find natural enemies of mealy bug.

NO. 13

DEPARTEMEN PERTANIAN
DIREKTORAT JENDERAL KEHUTANAN
PROYEK PEMBINAAN PERSUTERAAN ALAM
SULAWESI SELATAN

SILKWORM DISEASE IN INDONESIA
-REAL CONDITION AND CONTROL-

BY

DR. HAJIME INOUE

SERICULTURE DEVELOPMENT
COOPERATION PROJECT
(ATA - 72)
MAY, 1980

P R E F A C E

The author joined to the Sericulture Development Cooperation Project in Indonesia (ATA-72) based on the agreement between Japanese and Indonesian Governments, and has been stayed in the district of the South Sulawesi for two years from June 20, 1978 to June 19, 1980 as an Expert of the control of disease and pest of silkworm and mulberry.

This is the summary of survey and investigation of the silkworm disease performed with the counterparts and assistant counterparts at the Subcenter, Soppeng Prefecture. The members of research team are as follows :

Dr. HAJIME INOUE	(Expert)
Mr. HATTA MADJID	(Counter Part)
Mr. Ir. ACHMAD ANWAR	(Counter Part)
Mr. EDDY HARYADI	(Assistant Counterpart)
Mr. BASIR KAMARUDDIN	(Assistant Counterpart).

Until the present, reports on the silkworm disease are scaresely published in Indonesia. Therefore, this report may be believed to contribute for the development of sericulture in this nation.

The author wishes to express his thanks to Mr.Ir.J. Suripto, the head of this Project, and Dr. N. Mori, the leader of Japanese team, for their encouragement throughout the work.

Thanks are .....

Thanks are due to all of persons who are concerned to this Project for their invaluable assistance.

Bili - Bili, May 1980.-

H. Inoue

(Dr. HAJIME INOUE)

C O N T E N T S

I N T R O D U C T I O N

The major district of sericulture in Indonesia is the South Sulawesi and sericulture farmers are distributed especially at prefectures of Soppeng, Wajo, Sidrap and Enrekang.The history of sericulture in this district is comparatively new and it is said that it occurred in around 1964. The scale of silkworm rearing is small and the larvae of 0,5 to 1 box is reared under the floor of "high-leg" farmer's house. In the first time, a polyvoltine race was used, but owing to the big scale danage by pebrine disease, Indonesian Government prohibited to self-egg raising of polyvoltine by farmers in 1974. At present, an imported F_1 silkworm and its domestic F_2 silkworm are prepared for silkworm rearing. The young silkworm of 1st to 3rd instar is reared at "Unit (so-called cooperative - rearing house)" and then the grown silkworm of 4th instar is delivered to farmer's house. In the major sericulture place, three prefectures of Soppeng, Wajo and Sidrap are located at a flat ground-high temperature place, that is, it is 120 m - height from sea level and its temperature is above $30^{\circ}C$, resulting the silkworm larvae make cocoon at 20 days later. On the other hand, Enrekang prefecture is located at a north-mountains place of 600 - 800 m height, and the larvae start to making cocoon at 23 - 24 days later. The silkworm rearing at this district was markedly damaged by pebrine disease in 1972, and then Indonesian Government requested a technical aid of sericulture to Japanese Government. According to the Agreement

concluded in 1978, the auther was dispatched to this nation.

The reports on the silkworm disease in Indonesia are few till now. Katsumata (1975), who had been at Bogor from 1965 to 1967, described that the damage by pebrine disease was big, the nuclear polyhedrosis virus disease was frequently observed, the fungus disease was few and only green muscardine was there, and the poisaning of tobacco gave a big damage to silkworm rearing. The preliminary survey team of Japan (1974) reported that the pebrine disease, nuclear polyhedrosis virus desease, and fungus disease including Aspergillus Sp., yellow and black muscardine, are observed in the South Sulawesi. At that time, the pebrine disease was very many and the pebrine spore was observed not only in the larvae from self-raised - egg by farmers, but only in the egg produced at the branch of Sericulture Station, Soppeng. The other disease except pebrine was not seemed to be a big problem, but Aspergillus disease had a possibility to became big because of its existence in the bamboo tools of silkworm rearing.

As mentioned above, the knowledge of silkworm disease in the South Sulawesi was quite few and our work was first focussed to clear the real condition of silkworm disease in this district, followed with the development of control techniques fitted to this district.

The research method and several tests adopted in our research are described in another book entitled "Research - techniques in the control of disease and pest of silworm and mulberry" (Inoue, 1980).

I. PATHOLOGICAL SURVEY ON SILKWORM DISEASE

In order to know the kind of silkworm disease and its real condition, a pathogenical survey was performed on the F_1 and F_2 larvae reared by farmer, and also the dust from their silkworm rearing places was examined by a bioassay method to detect pathogens.

I.1. Inspection of Sluggished Larvae at the Subcenter and Farmer

The sluggished larvae in the rearing tray are, in general, infected with pathogens, therefore, the sluggished larvae were collected and disected at the laboratory, and then examined with an Olympus BH microscope.

Primarily, the result of inspection on sluggished F_2 larvae from two farmers at Soppeng was as presented in Table 1.

Table 1. Inspection of Sluggished F_2 Larvae at Soppeng

No.	Place	Unit belonged	No. of larvae examined	Pathogen: NPV	CPV	Pebrine	Fungus	Others
I.	Subcenter		10	0	1	4	2	3
	Farmer A	23	8	3	1	0	0	4
	Farmer B	30	8	1	4	8	0	0
II.	Subcenter		20	0	0	1	20	0
	Farmer A	23	5	1	0	1	0	3
	Farmer B	30	5	0	0	5	0	0
	Farmer C	9	5	2	0	2	0	2

- Examination date : (I) July, 1978; (II) December, 1978.
- Silkworm race : Kinshu x Showa; 2 to 3 days of 5th instar.

The inspection was performed in the dry and rainy seasons. Some larvae showed no or very little body fluid. When that larvae examined in detail, fungus fibers were observed, and then such larvae was thought as Infection of fungus disease. The larvae wich showed difficulty in detection of pathogen with a light microscope was classified into "others". From diseased F_2 larvae, pathogens of pebrine, nuclear polyhedrosis virus (NPV), cytoplasmic polyhedrosis virus (CPV), and fungus were observed.

Next, the inspection result on sluggished F_1 larvae, imported from Japan, at Soppeng and Wajo was as presented in Table 2.

Table 2. Inspection of Sluggished F_1 Larvae at Soppeng and Wajo

Place	Unit belonged	No. of larvae examined	Pathogen: NPV	CPV	Pebrine	Fungus	Others
-Kabupaten Soppeng:							
Subcenter		10	6	0	0	3	1
Project Tanah BellangE		5	0	0	0	0	5
Farmer A	23	5	0	0	0	3	2
Farmer B	30	8	0	0	5	3	0
Farmer C	9	8	0	1	0	6	1
Farmer D	Mede	10	0	0	0	10	0
- Kabupaten Wajo :							
Farmer A	7	5	0	0	1	0	4
Farmer B	7	5	1	0	0	2	2

(August, 1978).-

- Silkworm race: Kinsyu x Showa; 3 to 4 days of 5th instar.

From the diseased F_1 larvae, NPV, CPV, pebrine and Fungus disease were observed.

When the data of F_1 and F_2 larvae were compared, the pebrine spore was more frequently found in F_2 larvae. This Sujested that the pathogen of pebrine might still come through eggs. The pebrine spore was also found in F_1 larvae, suggesting that pebrine spore was still existing at the silkworm rearing place. This thought may be supported with Farmer B (a same farmer) at Soppeng in Table 1 and Table 2.

I.2. Inspection of Dead Silkworm Cocoon from Farmer

Many cocoons of farmers were dead. In order to Prelimi nally know the reason why so many cocoon died, dead cocoons of F_1 were collected at random from farmers. The result of its inspection was as presented in Table3.

Table 3. Inspection of Dead Silkworm Cocoon from Farmer

Place	Unit belonged	No.of dead cocoon examined	Pathogen: Polyhedron	Pebrine	Fungus	Others
Farmer A	9	20	3	0	11	6
Farmer B	9	20	2	0	6	12
Farmer C	9	20	0	0	12	8
Farmer D	9	20	1	0	14	5
Farmer E	2	20	0	0	5	15

(Hatta M., March, 1979).

Pebrine spore was not found, but polyhedron and fungus were found. As to polyhedron, the tissue of pupae infected polyhedra was already dissolved, and this polyhedron was determinded as that of NPV. As to fungus disease, almost of

all were by Aspergillus infection.

I.3. Distribution of Pathogen at Silkworm Rearing Place of Project and Farmer

In order to know the contamination of silkworm rearing places of the Project and farmer with pathogens, the dust was collected from every rearing place, mainly at the floor. These dust were supplied to a bioassay method, that is, a small volume of sterilized water was added to the dust, and it was smeared on a mulberry leaf, and then the leaf was eaten by newly hatched larvae of C-108 x N-115 silkworm strain for one day. The larvae were reared for 12 days and dead larvae were examined. On the final day, all larvae were disected for the inspection of pebrine spore. The result was as presented in Table 4 (page 7). As clarified in the table, the dust from not a few Units and farmers showed a very strong pathogenisty. In many cases, it was due to NPV. The silkworm rearing places of the Project, though they were disinfected with formalin spray, pathogens were still existence in the rearing room. Especially at Project Enrekang, the dust of rearing room for young silkworm showed no pathogenisity, but the dust of rearing room for grown silkworm revealed a strong pathogenisity. This fact suggested that the pathogen was accumulated in the place in accordance with the growth of larvae. The pebrine spore was not detected in this survey performed in September, 1979. This may be due to the improvement of inspection technique in F_2 egg production.

Table 4. Bioassay of Dust from Silkworm Rearing Place

Place	No.of larvae tested	No.of healthy larvae	No.of dead larvae	Pathogen: Polyhedron	Pathogen: Pebrine	Pathogen: Fungus	Pathogen: Others
Control	10	10	0	0	0	0	0
Kabupaten Wajo :							
Project Wajo	10	5	5	3	0	2	0
Farmer A	10	10	0	0	0	0	0
Farmer B	10	5	5	5	0	0	0
Unit PT Kebun Ternak	10	10	0	0	0	0	0
Farmer C	10	1	9	9	0	0	0
Farmer D	10	9	1	0	0	1	0
Farmer E	10	8	2	0	0	2	0
Unit Sering Raya							
Farmer F	10	10	0	0	0	0	0
Farmer G	10	9	1	1	0	0	0
Farmer H	10	10	0	0	0	0	0
Unit CV Daya Murni							
Farmer I	10	10	0	0	0	0	0
Farmer J	10	10	0	0	0	0	0
Farmer K	10	10	0	0	0	0	0
Kabupaten Sidrap :							
Project Masseppe	10	9	1	0	0	1	0
Farmer A	10	9	1	1	0	0	0
Farmer B	10	10	0	0	0	0	0
Unit Social	10	10	0	0	0	0	0
Farmer C	10	2	8	8	0	0	0
Farmer D	10	10	0	0	0	0	0
Kabupaten Enrekang							
Project (Young larvae)	10	10	0	0	0	0	0
Project (Grown larvae)	10	0	10	10	0	0	0
Unit Belajen	10	9	1	0	0	1	0
Farmer A	10	10	0	0	0	0	0
Farmer B	10	0	10	9	0	1	0
Farmer C	10	8	2	1	0	1	0
Unit Sudu	10	9	1	0	0	1	0
Farmer A	10	9	1	0	0	0	1
Farmer B	10	9	1	0	0	1	0
Farmer C	10	10	0	0	0	0	0

(September, 1979).-

I.4. Real Condition of Nuclear Polyhedrosis Virus Disease

The larvae infected with nuclear polyhedrosis virus (NPV) are frequently observed in the silkworm rearing tray of farmer. In addition, NPV is existence in the Units and farmers as shown in Table 4. Therefore, this examination was perform-ed to get information of appearance NPV-infected larvae in the tray. The abnormal 20 larvae of 3rd instar from a Unit and also each 20 larvae of 4th and 5th instar of a farmer were collected at random, and then the existence of polyhedron of NPV in the body fluid was examined with a light microscope.

The result of 4-th (or 5-th) instar larvae presented in Fig. 1 was the total of 60 larvae from 3 farmers belonging to same Unit. The result was as presented in Fig.1. In any abnormal 3rd instar larvae from the Unit, NPV was not observed, but on the contrary NPV was found in the abnormal 4th instar-larvae of 2 to 3 days old, and its finding frequency increased in the abnormal 5th instar larvae of 3 to 4 days old. Although it was not clear that when these larvae infected with NPV, the dust from Unit CV Nurwena included NPV.

I.5. Search of Flacherie Virus

A flacherie virus disease is the most important silkworm disease in Japan owing to its strong infectivity. The search on the existence or not of flacherie virus (FV) is now continued mainly with the staining method of Unna-Pappenhaim solution. Until now, FV has not been found in the South Sulawesi.

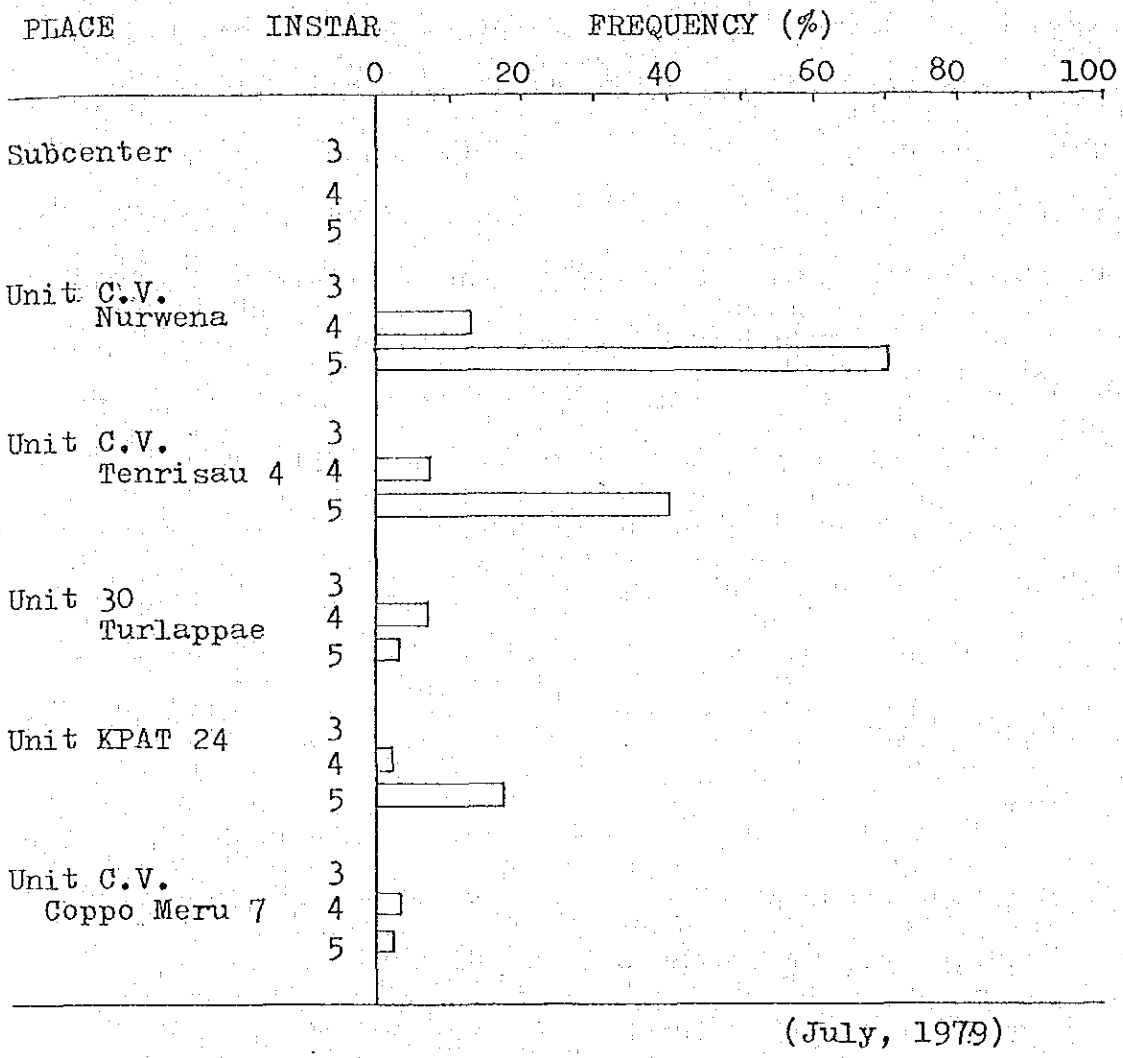


Fig. 1. Appearance of NPV-Infected Larvae in the Rearing Tray.

I.6. Discussion

The result of this primary survey on the silkworm disease revealed that NPV, CPV, Pebrine and *Aspergillus* Sp. were the main cause of silkworm disease. Moreover, white muscardine, green muscardine, yellow muscardine and a bacteria of *Serattia* group were found, though they were very few and their

nature was not exactly examined. This was the first report of CPV in Indonesia as well as in the South Sulawesi. Although CPV was frequently observed, the damage caused by CPV was seemed to be the low grade at present. FV was not found until now, but FV gave a great damage to the sericulture farmer in Brazil besides Japan (Oohata, 1976, Personal Communication) and FV was in China (Watanabe, 1979, Personal Communication) and Iran (Yamazaki, 1975), and the survey must be continued.

Some larvae of 5th instar showed an unusual symptom - such as a part of larval body became thim (See-through). A fat body of such larvae was destroyed. The disease like this is not reported in Japan, and it seemed to be a specific disease in Indonesia.

The result of this primary survey suggested that the Aspergillus disease and NPV disease are the most important diseases at present. As to the pebrine disease, in the F_1 and F_2 larvae of farmer A at Soppeng (Table 1 and Table 2), who is the same farmer, pebrine-infected larvae were observed, suggesting that pebrine spore was still at the farmer's house.

II. INSPECTION OF DEAD SILKWORM COCOON

In order to know more in detail the disease of grown larval stage, the dead silkworm cocoons, which were reared for F_2 egg production, were examined. From F_1 cocoons from the Project and consignment farmers, a group of 50 cocoons was collected at randum from every Project or farmer. The cocoon was disected to separate healthy and dead pupae (or larvae). All the pupae were examined for the detection of pebrine infection, but only dead pupae were examined for other pathogens.

II.1. Dead Cocoon in Wet Season

Table 5 (page 12) showes the inspection result of F_1 cocoons in the wet season. The mortality percent was very high on the whole and 4 rearing places showed the mortality of 50% above. The pebrine spore was not found and the main cause of the death was the Aspergillus disease and NPV disease. After this survey, the same survey was performed at the end of March, and was obtained the similar data. Moreover, the result in Table 5 was almost equal with the result of farmer's dead cocoon at Soppeng (Table 3). Thus the Aspergillus disease gave a marked damage to the farmer in the wet season.

II.2. Dead Cocoon in Dry Season

Table 6 (page 13) showes the inspection result of F_1 cocoon in the dry season. The mortality percent was very high even though the dry season and 2 rearing places showed mortality of 50% above. The Aspergillus disease was comparatively

Table 5. Dead Cocoon in Wet Season

Place	No. of cocoon examined	No. of healthy cocoon	No. of dead cocoon	Mortality percent (%)	Pathogen: Poly hedran	Pathogen: Pebrine	Pathogen: Fungus	Pathogen: Others	Remarks
-Kabupaten Soppeng									
Subcenter	50	35	15	30	4	0	3	8	
Project Tanah BellangE	50	42	8	16	0	0	6	2	Aspergillus
-Kabupaten Wajo									
Project Wajo	50	13	37	74	4	0	25	8	Aspergillus
Farmer A	50	43	7	14	0	0	5	2	"
Farmer B	50	38	12	24	1	0	6	5	"
Farmer C	50	14	36	72	28	0	8	0	N P V
-Kabupaten Sidrap									
Project Masseppe	50	20	30	60	0	0	28	2	Aspergillus
" DattaE	50	15	35	70	0	0	31	4	"
Farmer A	50	38	12	24	5	0	5	2	"
Farmer B	50	31	19	38	0	0	11	8	"
Farmer C	50	45	5	10	1	0	2	2	
Farmer D	50	41	9	18	2	0	1	6	
Farmer E	50	32	18	36	0	0	9	9	Aspergillus
-Kabupaten Enrekang									
Project Enrekang	50	44	6	12	0	0	2	4	
Project S. Dollok	50	48	2	4	0	0	1	1	
Project S u d u	50	49	1	2	0	0	0	1	

(January, 1979).-

many and also NPV disease. But in many case, the diagnosis by only disection faced with difficulty.

II.3. Discussion

The purpose of examination of dead silkworm cocoons was to guess the infection of pathogens at the larval stage of 5th instar from the pathological inspection of dead cocoons. The result revealed that the main causes were the Aspergillus and NPV disease. The result was quite similar to the data in Thailand (Sugiyama, 1980). That is, in Thailand cocoons from farmers at reclamation of wasteland were highly infected with Aspergillus Sp.

Table 6. Dead Cocoon in Dry Season

Place	No.of Cocoon examined	No.of healthy cocoon	No.of dead cocoon	Mortality percent (%)	Pathogen: Polyhedrone	Pathogen: Pebrine	Pathogen: Fungus	Pathogen: Others
Soppeng								
Subcenter	50	45	5	10	0	0	0	5
Proj.Tnh Bellang	50	29	21	42	1	0	2	18
Wajo								
Project Wajo	50	20	30	60	7	0	9	14
Farmer A	50	1	49	98	4	0	8	37
Sidrap								
Project Massepe	50	39	11	22	0	o	4	7
Project DataF	50	44	6	12	0	0	2	4
Farmer A	50	38	12	24	0	0	2	10
Farmer B	50	42	8	16	0	0	0	8
Farmer C	50	44	6	12	0	0	1	5

(Achmad A.; August, 1979).-

III. REAL CONDITION OF PEBRINE DISEASE

In the past year, pebrine disease markedly spreaded with the cause of self-egg raising of polyvoltine race by farmers. After the time, however, the farmer's self-egg raising was prohibited and imported F_1 and its F_2 eggs were used. Herewith, the survey of real condition of pebrine disease was performed.

III.1. Pebrine Infection of Farmer's Silkworm Larvae

In order to know the pebrine infection of farmer's silkworm larvae, their larvae were examined for 4 months from April to July, 1979. The research method was that one unit and three farmers belonging the Unit were choiced, and then 20 larvae of 3rd instar and each 20 larvae of 4th and 5th instar, especially sluggished larvae, were obtained from the Unit and farmer, respectively. The larvae obtained were disected and inspected the existence of pebrine spore with the light microscope.

III.1.1. Inspection of F_1 Larvae

The F_1 eggs from Japan was ones passed the pebrine inspection, and if pebrine infection of farmer's F_1 larvae is examined, we can learn the pebrine infection at farmer's silkworm rearing places. The result of survey at Soppeng was as presented in Table 7. (page 15). From 3rd instar larvae of Units was not found pebrine spore, but found in the 5th instar larvae of the Subcenter and farmers. Especially, the pe-

brine spore was found in the larvae of all farmers belonging to the unit 30, and this fact suggested the possibility of pebrine infection at the unit.

Table 7. Pebrine Inspection of F_1 Larvae at Soppeng

Place	3-rd instar		4-th instar		5-th instar	
	+	-	+	-	+	-
Subcenter	0	20	0	20	1	19
Unit 4 SoliE	0	20				
Farmer A			0	20	0	20
Farmer B			0	20	0	20
Farmer C			0	20	0	20
Unit 9 Pissing	0	20				
Farmer A			0	20	0	20
Farmer B			0	20	0	20
Farmer C			0	20	0	20
Unit 30 TurlapaE	0	20				
Farmer A			0	20	3	17
Farmer B			0	20	4	16
Farmer C			0	20	2	18

+ : pebrine infection - : non pebrine infection

III.1.2. Inspection of F_2 Larvae

The inspection result of F_2 larvae, which eggs were produced by the project, was as presented in Table 8. (page 16). On the contrary in the case of F_1 larvae, pebrine-infected larvae were appeared in the 3rd instar larvae at units as well as in the 4th and 5th instar larvae.

Furthermore, the inspection of F_2 larvae was continued as presented in Table 9 (page 16). The number of pebrine-infected larvae was a few at this time. The farmer A belonging to unit 30 TurlappaE showed many diseased larvae in the first survey (Tabel 8) but no infected larvae in the second survey (tabel 9).

Table 8. Pebrine Inspection of F_2 Larvae at Soppeng (1)

Place	3rd instar +	3rd instar -	4th instar +	4th instar -	5th instar +	5th instar -
Subcenter	2	18	3	17	2	18
Unit 30 TurlappaE	3	17				
Farmer A			3	17	4	16
Farmer B			1	19	2	18
Farmer E			3	17	2	18
Unit 7 Ukke	0	20				
Farmer A			0	20	3	17
Unit 10 Solie	0	20				
Farmer A			7	13	2	18
Unit 11 Ukke	1	19				
Farmer A			1	19	2	18

Table 9. Pebrine Inspection of F_2 Larvae at Soppeng (2)

Place	3rd instar +	3rd instar -	4th instar +	4th instar -	5th instar +	5th instar -
Subcenter	0	20	0	20	0	20
Unit 30 TurlappaE	0	20				
Farmar A			0	20	0	20
Farmer D			0	20	0	20
Farmer F			0	20	0	20
Unit CV Nurwena	0	20				
Farmer A			0	20	0	20
Farmer B			1	19	3	17
Farmer C			0	20	0	20
Unit CV Tenrisau 04	0	20				
Farmer D			0	20	0	20
Farmer E			1	19	0	20
Unit KPAT 24	0	20				
Farmer A			0	20	1	19
Farmer B			0	20	0	20
Farmer C			0	20	0	20
Unit CV Coppo Meru 07	0	20				
Farmer A			0	20	0	20
Farmer B			0	20	0	20

This fact suggested that the pebrine spore in F_2 larvae of farmers might be derived from eggs in addition to the infection at earlier stage of larvae at Units.

III.2. Pebrine Infection of F_1 and F_2 Larvae Reared at the Same Time

The F_2 larvae were reared by a few farmers at the rearing time of F_1 larvae imported from Japan. We collected several sluggished larvae from F_1 and F_2 rearing places and examined pebrine infection. The result was as presented in Table 10 (page 18). As apparent in the table, there was no pebrine infected larvae in the F_1 larvae, but on the contrary, almost all of F_2 larvae, such as 60-100%, were infected with pebrine. It was very interested that the Farmer A belonging to Unit 23, Soppeng, reared F_1 and F_2 larvae at the same place, but the pebrine spore was only found from F_2 larvae. These result revealed that this pebrine spore was derived from silkworm eggs. According to the followed survey, we knew that this F_2 egg was not one produced at the Subcenter.

III.3. Distribution of Pebrine Spore at the Silkworm Rearing Place of Farmer

In order to know the distribution of pebrine spore, the dust of silkworm rearing place of farmer and Units was collected and examined its pathogenisity by the biossay method. The result waa as presented in Table 11 (page 19). By the inoculation of dust, several diseased larvae appeared, but pebrine-infected larvae were quite a few. This result was quite similar

to the result in Table 4. However, the dust of Unit 30, where usually observed pebrine infected larvae (Table 7 and Table8), included pebrine spores.

Table 10. Pebrine Infection of F_1 and F_2 Larvae Reared at the Same Time

Place	Kind of Silkworm	No.of larvae examined	No. of diseased larvae	Percent of diseased larvae (%)
Soppeng				
Subcenter	F_1	5	0	0
Farmer A (Unit-23)	F_1	10	0	0
Farmer A (")	F_2	8	8	100
Farmer D (Unit 9)	F_1	10	0	0
Sidrap				
Project Massepe	F_1	10	0	0
Enrekang				
Project Enrekang	F_1	10	0	0
Farmer A	F_2	10	8	80
Farmer B	F_2	10	6	60
Farmer C	F_1	10	0	0

- Silkworm larvae : 4th instar larvae of 2-3 days old.

III.4. Hatching of Larvae from Eggs Raised by Pebrine-Infected Female Moth

The hatching rate of larvae from eggs raised by pebrine-infected female moths was examined. In their female moths, there were very many pebrine spores. The F_2 egg-mass raised by one female moth was devided into 4 blocks, and only one block was prepared for the examination. The result was as presented ir Table 12 (page 20). The hatching of larvae was comparatively good and its average was 95,1 %.

Table 11. Distribution of Pebrine Spore at Farmers in Soppeng

Place	No.of larvae tested	No.of healthy larvae	No. of diseased larvae	No.of pebrine larvae
Control	10	8	2	0
Unit 07 Ukke	10	3	7	1
Farmer A	10	0	10	0
Unit 08 Solie	10	4	6	0
Unit 09 Pissing	10	8	2	0
Farmer A	10	8	2	0
Farmer E	10	2	8	0
Farmer F	10	4	6	0
Unit 10 Solie	10	5	5	0
Farmer A	10	4	6	0
Unit 11 Ukke	10	0	10	0
Unit 30 TurlappaE	10	1	9	1
Farmer A	10	8	2	0
Farmer E	10	6	4	0
CV Nurwena	10	0	10	0
Farmer D	10	2	8	0
Farmer E	10	0	10	0
Unit KPAT 08	10	0	10	0
Farmer A	10	1	9	0
Unit 04 Sollie	10	3	7	0
Farmer A	10	0	10	0
Farmer A (Unit 02)	10	6	4	0

(June, 1979).-

- The newly hatched larvae of F_1 were used and reared for 12 days.

III.5. Trans-Ovum Transmission of Pebrine in the Larvae Raised by Pebrine-infected Female moth

The eggs raised by pebrine-infected or non-infected female moths were examined about the trans-ovum transmission. - Every 3 egg-masses was choiced from pebrine (+) and (-) eggs - and one egg-mass was devided into 4 blocks. Only one block of

Table 12. Hatching Percent of Eggs Raised by Pebrine-infected Moth

No. of moth	No. of eggs	No. of larvae harched	Hatching percent (%)
1.	84	83	98,8
2.	120	116	96,7
3.	128	123	96,1
4.	134	123	91.8
5.	158	156	98,7
6.	134	128	95,5
7.	141	130	92,2
8.	100	95	95,0
9.	141	129	91,5
Average			95,1

(April, 1979).-

them was prepared for the inspection. The larvae from one - block were reared in a petri dish, and on day 3 and 8 after the hatching every five larvae was obtained at random, and then disected for the inspection. The result was as presented in Table 13 (page 21). On day 3 after the hatching, almost all of larvae from Pebrine (+) possessed very many spores,and also on day 8. On the contrary no larvae from pebrine (-) - showed the existence of spore in the body.

III.6. Time of Death of F_2 Larvae Deribed from Pebrine-Insfected Female Moth

The newly hatched F_2 larvae from eggs raised by pebrine infected female moth were reared in order to know the time of

death. Twenty egg-masses raised by 20 female moths were prepared. A group of 20 larvae was choiced at random from one egg-mass and reared in a petri dish. The dead larvae were taken off from the petri dish every day. The cumulative mortality -

Table 13. Inspection of Pebrine Spore in the Larvae from Pebrine (+) and (-) Female Moths

Pebrine	No. of egg mass	Existence of Spore On day 3					On day 8				
+	1	+++	++	++	+++	+++	+++	+++	+++	+++	+++
	2	++	—	++	—	—	+++	+++	+++	+++	+++
	3	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
-	1	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-

- Grade : +++ > ++ > + > - (September, 1978).-

percent were summarized about total 400 larvae from 20 egg-masses as presented in Fig.2. (page 22). In the period of 1st to 3rd instar, about 10 percent of larvae died, but the other grew into 4th instar same as control larvae. After the 4th instar, however, the growth of larvae was not the same and many larvae died in the 4th and 5th instar. The larvae of 39 percent became mature larvae, though the larvae delayed in growth one to two days when it was compared with the control larvae. But this larvae could not make cocoons.

III.7. Pebrine .....