インドネシア養蚕開発計画

専門家報告書

THE REPORT OF JAPANESE EXPERTS FOR THE SERICULTURAL DEVELOPMENT PROJECT IN INDONESIA

病虫害防除
① HISTOLOGICAL TECHNIQUES FOR DIAGNOSIS OF SILKWORM DISEASES
② CONTROL METHOD OF PEST INSECTS IN MULBERRY FIELD

桑害虫防除
MULBERRY INSECT PEST CONTROL

土壌

昭和58年2月
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国際協力事業団
JAPAN INTERNATIONAL COOPERATION AGENCY

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インドネシア養蚕開発計画は，昭和51年3月30日に署名された討議々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日本・インドネシア両国の関係機関の努力により大きな成果を収めております。

本報告書は，「病虫害防除」について阿部芳彦専門家（派遣期間：昭和55年6月6日～昭和57年6月5日），「桑害虫防除」について菊地　実専門家（派遣期間：昭和56年9月2日～同年12月1日），「土壌」について早坂　猛専門家（派遣期間：昭和57年3月26日～同年6月25日）の貴重な成果を取りまとめたものであり，今後の技術協力に携わる方々に大いに活用されることを願うものであります。

終りに，この報告書をとりまとめられた専門家の方々のご努力に感謝申し上げると共に本プロジェクトの一層の発展を期待する次第であります。

昭和58年2月

国際協力事業団
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村田稔尚

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PART II CONTROL METHOD OF PEST INSECTS IN MULBERRY FIELD

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派遣期間

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PART I

HISTOLOGICAL TECHNIQUES FOR DIAGNOSIS OF SILKWORM DISEASES

BY

YOSHIHIKO ABE

I. INTRODUCTION

1. Histological technique.

Primitive technique in histology is microscopic observation without any treatment; dissect a certain piece of tissues from organisms and put the piece on slide glass and mount with cover glass, and observe with microscope. Although the method is very simple and we can observe real condition of tissues and cells, the informations obtainable from microscopic fields by the method is not so much, and tissues and cells alter immeddiately their nature and form during observation. To improve these conditions, fixing and staining techniques of tissues and cells were developed and established as a routine work in Biology and Medical science.

2. Histology as a tool of diagnosis.

External symptom is an indicater to diagnose silkworm diseases; Mycosis and Nuclear polyhedrosis for example. However, we can not diagnose exactly the diseases by observation of external symptom alone, because the external symptom of silkworm diseases appears at crisis, and Protozoasis, Bacteriosis and some viral diseases have no clear external symptom comparable in each other. In such a case, we must introduct histological methods to diagnose exactly the diseases.

3. Histopathology.

Infection of microorganisms causes various reactions on host organisms, degeneration or decomposition of organs, tissues or cells, accumulation of foreign substances in tissues or in cells, inflammatory response, etc. Therefore we can detect the disease doing comparative observation between infected tissues and healthy ones if we know well interactions between host and parasite. This technique can be said to find out

abnormality of organs, tissues and cells in host organism caused by parasitic organisms. To find out abnormality of organs, we must know well normal condition of organs, tissues and cells in host. The histopathology is one of comparative histology.

In the text, the author picked up some basic techniques of histology for diagnosis of silkworm diseases.

II. SMEAR PREPARATION.

1. Smear preparation.

Smear preparation is very simple method to observe tissues and cells. A piece of tissue or a drop of blood is put on a slide glass and smear using spatula, or squash with cover slip or spread with an edge of cover slip or fine glass needle. Rapid examination can be done by the method.

2. Materials.

To dissect the tissues, some basic instruments are required; bottle for anaesthetization, instruments for anatomy, etc.

a. Bottle for anaesthetization.

Deep cylinder bottle with coverture is required for anaesthetization(Fig. 1). In the bottle, a large piece of absorbent cotton is put in, and some drop of ethyl ether are poured. Thereafter the cotton is covered with thin cork plate and paraffin paper. The silkworm is put on the paper and anaesthetized.

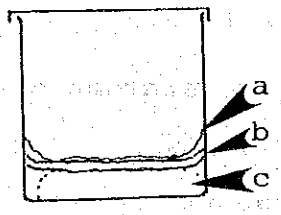

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Fig. 1. Bottle for anaesthetization.
a: Paraffin paper.
b: Cork plate.
c: Absorbent cotton cotaining ethyl ether.

b. Dish for anatomy.

Rectangular dish(about 12 X 8 cm) with cork or rubber sheet

is useful(Fig. 2). If there is no dish mentioned above, pour melted paraffin in petri dish(about 13 cm in diameter) and use the dish after paraffin become solid.

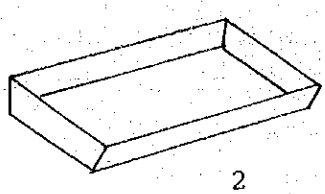

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Fig. 2: Dish for anatomy.

c. Needle for firming the animal an anatomy dish.

Insect pin is available for firming the test animals on anatomy dish. Fine injection needle are also available if used one is obtainable.

d. Pincettes and scissors for anatomy.

Fine small ones are usefull for anatomy of the insects. These for Opthalmology are available(Fig. 3).

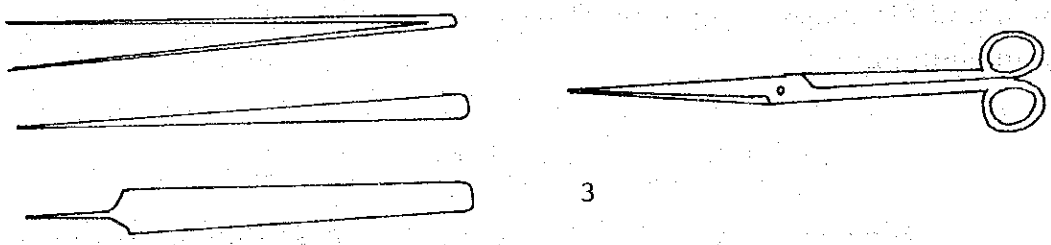

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Fig. 3: Fine pincettes and scissors for anatomy of the silkworm.

3. Smear of blood(hemolymph).

Before starting the smear, slide glass must be kept clean. Wash well using cleaner, wash well with water after cleaner, pass in distiled water, and stock in ethyl ether containing

alcohol. Before using, sweep well the glass using clean gauze and dry well. Cover slips must also be treated as mentioned above.

Smear of blood of small silkworm larvae as first or second instar is difficult. One of method is dissection of larval skin on slide glass and run out a drop of blood onthe glass, and cover the blood drop with another slide glass or cover slip, then remove out covered one immediately after bllod spread on the glass, thereafter dry blood film quikly exposuring to wind from electic fan. Electric hair dryer is practic for drying the blood film.

For large larva like as third to fifth instar, cut a dorsal leg of larva and put a drop of blood on a terminal portion of a slide glass, and spread blood drop using an edge of cover slip, another slide glass or fine glass needle as is shown in Fig. 4.

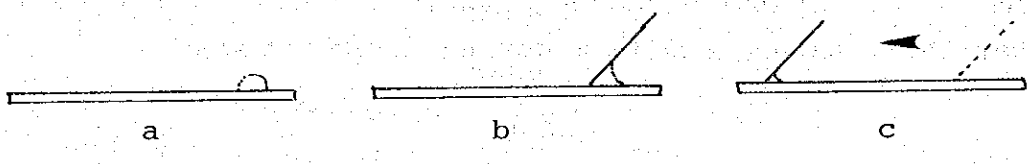

Fig. 4: Smear method of blood.
a: Put a drop of blood on a terminal of slide glass.
b: Tuch an edge of cover slip on a side of blood drop.
c: Push the cover slip quikly to another side of terminal portion of the slide glass, then dry quikly spreaded blood film.

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Pipettes of Pasteur is available for smear of blood. Tip of pipette is heated and pulled to make a fine end(Fig. 5).

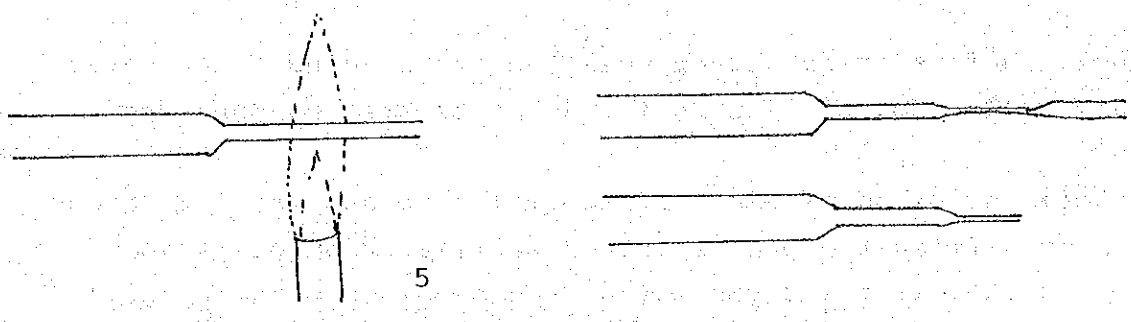

Fig. 5: Making fine end of pipette.
a: Heat a tip of pipette to melt.
b: Pull an end of pipette using pincettes for elongation of melted portion.
c: Cut off an end of tip.

Insert fine tip of pipette in hemocoel from segment membrane, then pull off when blood sream enters in cavity of pipette, and put a drop of blood on a terminal portion of slide glass(Fig. 6). The method can prevent excess dropping of the blood on the glass. Excess blood dropping makes thicker blood film to fall staining and observation, because blood cells alter their nature and form during drying procedure.

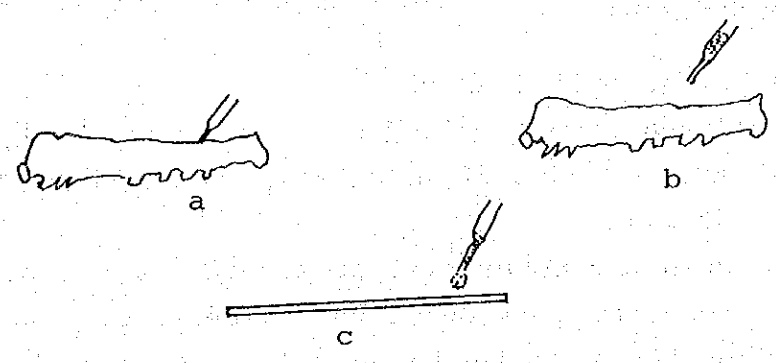

Fig. 6: Smear method using pipette of Pasteur.
a: Insert fine tip of pipette into hemocoel from segment.
b: Bring a certain volume of blood from hemocoel.
c: Put a drop of blood on slide glass.

4. Another tissues.

Anaesthetized silkworm larva by ethyl ether about three minutes is firmed on anatomy dish with insect pins at caudal portion and head. Then, dissect hind skin of larva from caudal portion tward the head using fine scissors, htereafter open and spread skin of larva and firm on the dish as is shown in Fig. 7. Dissect a piece of tissue desired and put it on center of a slide glass, then cover the piece of tissue with another slide glass or cover slip and press later slide glass or cover slip to spread the tissue on the slide glass(Fig. 7). Remove out quikly the later slide glass or cover slip and dry spreaded tissue film immediately.

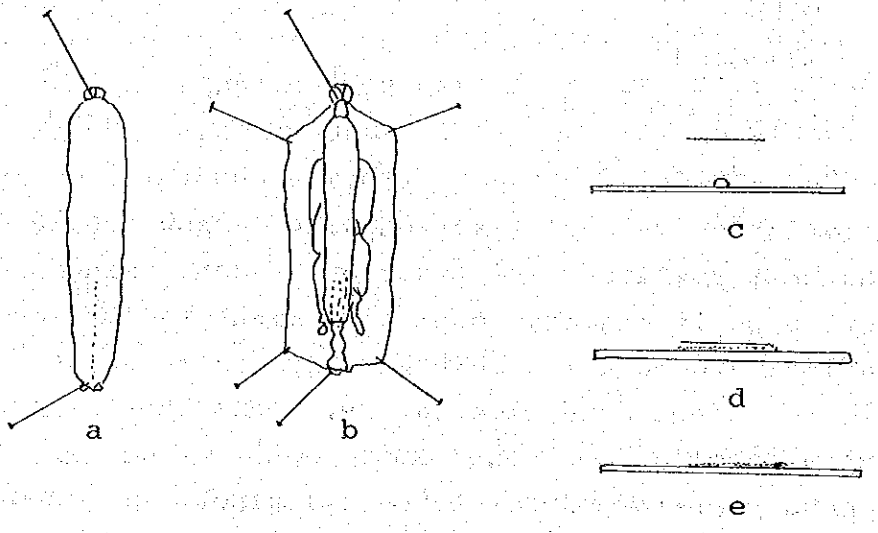

Fig. 7: Smear method of tissue.
- a: Firm anaesthetized larva on anatomy dish.
- b: Open skin of larva and bring a piece of desired tissue.
- c: Put the piece on a slide glass.
- d: Cover the piece with a cover slip and press the slip.
- e: Remove out quikly the slip and dry spreaded tissue film.

5. Fixation of smeared preparation.

To maintain good results and prevent alteration of substances in smeared tissues, some fixatives are better to applicate. When preparation must be stocked without staining, 80% ethyl alcohol is utilizable for stocking solution. The preparations may be stocked in staining bottle as in Fig. 8. For the

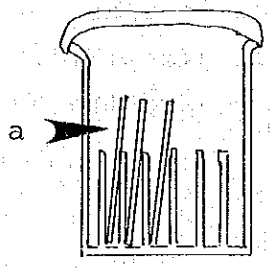

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Fig. 8: Stock and fixation method of ameared preparation.
a: Smeared slide glass.

Pyronine-methylgreen method, fixation with Carnoy's solution may give the best results. The fixation is done using staining bottle as in Fig. 8, about five minutes. Thereafter the preparations are washed with tap water using the bottle. If the staining is not done immediately, stock the preparations in 80% ethyl alcohol as mentioned above.

For Giemsa staining, absolute methyl alcohol is used as fixative. Put smeared preparatios in staining bottle containing absolute methyl alcohol several minutes and put out the preparations and dry inroom temperature. Onother method is, put smeared preparations on paralleled glass rods and pour absolute methyl alcohol on the preparation as smeared portion is covered enough with the fixative. Then dry the preparation in room temperature(Fig. 9).

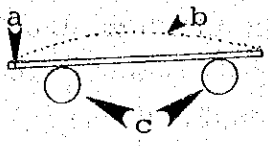

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Fig. 9: Fixation with absolute methyl alcohol.
a: Slide glass.
b: Poured absolute alcohol.
c: Glass rods as suspender of slide glass.

6. Staining methods of smeared preparations.

Giemsa staining is used generally for smeared samples. Another methods are May-Grunwald stain, Wright stain and combination of Giemsa and May-Grunwald or Wright stain. For diagnosis of Viral Fracherie, Pyronine-methyl green is well known.

a. Giemsa method.

1). Fix smeared and dryed preparation with absolute methyl alcohol.
2). Dry the fixative in room temperature.
3). Pour 20 - 40 times diluted Giemsa water solution(use distiled water), 15 - 20 minutes.
4). Wash well the preparation with distiled water.
5). Dry under room temperature and examin with low magnification light microscope(X 10 object lens), and if the results of staining are not sufficient, stain again as mentioned above.
6). After dry, examin using immersion oil lens.

Results: RNA rich cytoplasm is colored blue while necleus is colored as red. Acidophilic substance become red, basophilic substances are blue, neutral substances are purple red to purple blue.

b. May-Grunwald stain.

1). Fix smeared and dryed preparation with some drop of May-Grunwald solution less than 30 seconds.

2). Add equal volume of distiled water to the fixative for dilution, and stain the perparation with diluted fixative about 15 minutes(Fig. 10).

3). Wash well the preparation with distiled water.

4). Dry and examin using immersion lens.

Results: Same results may be obtained as Giemsa method. However, if the fixation is done so longer, accumulation and precipitation of dye grains give contamination on the preparation which interefares the observations.

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Fig. 10: Staining method for May-Grunwald and Wright.
a: Fix with each solution.
b: Dilute the solution and stain the preparation with diluted solution.

c. Wright stain.

1). Fix smeared and dryed preparation with some drop of Wright solution, 30 seconds to one minute.

2). Add equal amount of distiled water and stain the preparation with diluted fixative 15 - 20 minutes.

3). Wash well the preparation with distiled water.

4). Dry and examin as mentioned in former staining methods.

Results: Same results may be obtained as mentioned above. The method also gives contamination by dye grain as mentioned above.

d. May-Grunwald Giemsa stain.

1). Fix smeared and dryed preparation with some drops of May-Grunwald solution less than 30 seconds.

2). Dilute the fixative as mentioned above, and stain about five minutes.

3). Discard the diluted fixative and pour 20 times diluted Giemsa solution on the preparation and stain about 20 minutes.

4). Wash well with distiled water.

5). Dry and examin as mentioned above.

Results: Same as mentioned above, but obtainable the finest preparation.

e. Wright Giemsa Stain.

1). Fix smeared and dryed preparation with Wright solution within one minute.

2). Dilute the fixative as mentioned above, and stain about five minutes.

3). Discard the diluted fixative and pour 20 times diluted Giemsa solution and stain about 20 times.

4). Wash well with distiled water.

5). Dry and examin as mentioned above.

Results: Same as mentioned above, but obtainable the finest preparation. Namely, the method may be suitable for diagnosis of protozoasis.

f. Pyronine-methylgreen stain for basophilic bodies of Infectious fracherie virus.

1). Smear fore or middle portion of midgut epitherium from diseased larva.

2). Fix the preparation with Carnoy's fixative(pp. 14) three to five minutes.

3). Wash the fixed preparation with tap water about three minutes.

4). Stain with Pyronine-methylgreen solution(pp. 33).

5). Mount directly with cover slip and examin.

If parmanent mount preparation is required;

6). Rince slightly with distiled water.

7). Rince with tert-Butyl alcohol two times, 30 seconds in each time.

8). Pass xylene two times, three minutes in each time.

9). Mount using Balsam canadacum or Caedax, and examin.

Results: Nucleus is colored as blueish green while cytoplasm is colored as red. Basophilic bodies caused by Infectious fracherie virus are colored as deep red.

III. PRAFFIN SECTION METHOD.

The method is the most general in histology. Tissue desired to observe is embedded in solid paraffin and dissected mechanically as thin film which is transparent the light under microscopic field. Staining trchniques are also applied for differenciarion of tissue structure, cell structure and substances in cells and tissues.

1. Fixatives.

Purpose of fixation is to prevent alteration of tissue or cell structure and substances, and preserve soluble substances in cell and tissue. Fixatives useful for histology of the silkworm are as follows.

a. Methyl alcohol.

Absolute one is used for fixation of blood film on slide glass, smeared or crashed preparation as pretreatment for Giemsa staining as mentioned above.

b. Ethyl alcohol.

70% - 80% water solution is used generally for fixation and for stock of fixed tissue or smeared preparation.

c. Formalin.

Concnteration of commercial formalin is 37% - 40%. Generally, 10 times diluted water solution is used for fixation. Neutral buffered ones are also used. Lillie's method is as follows;

Formalin(commercial)	100 ml
Distiled water	900 ml
Sodium acid phosphate monohydrate	4.0 gr
Disodium phosphate, anhydrous	6.5 gr

The fixative is not penetrable in tissue so rapid that

long time is required for fixation. For larger block of tissue (more than three mm), it would bebetter fixing under cool condition to prevent autolysis of tissue or alteration of tissue; over night fixing in refrigerator for example.

d. Alcohol formalin solution.

Neutralized formalin	10 ml
95% ethyl alcohol	90 ml

Neutralized formalin: Add a good amount of Calcium carbonate in commercial formalin.

2 - 4 hours fixation is better for tissue of the silkworm.

e. Carnoy solution.

Absolute(or 95%) ethyl alcohol	60 ml
Chloroform	30 ml
Glacial acetic acid	10 ml

The fixative is penetrable so rapid in tissue that fixing time is less than two hours. Because tissue become very hard by long time fixation and tissue may be broken during sectioning after embeddind. One hour fixing is enough for all tissues of the silkworm.

2. Fixation of tissues.

Smaller larvae such as first and second instar are fixed directly in fixative. Carnoy solution may be suitable for such a case because the solution is penetrable rapidly in tissues. 20 minutes after in fixative, larvae must be dissected in small piece, and fixed again in new solution. If whole body section is required, several parts of skin must be cut to give slits for increasing penetrability of the solution in tissues, thereafter the larvae are put in new solution. Total time of fixation must be within one hour.

For grown silkworm larvae, anaesthetization is required. Thereafter anaesthetized larva is firmed on anatomy dish using pins by each end(head and caudal portion). Then skin is dissected from caudal portion to head. Skin is also firmed on the dish after dissection and opening, and fixative is poured on whole body of opened larva as the larva is dipped enough in fixative(Fig. 11). Twenty minutes after, the tissue desired must be dissected and put in new fixative.

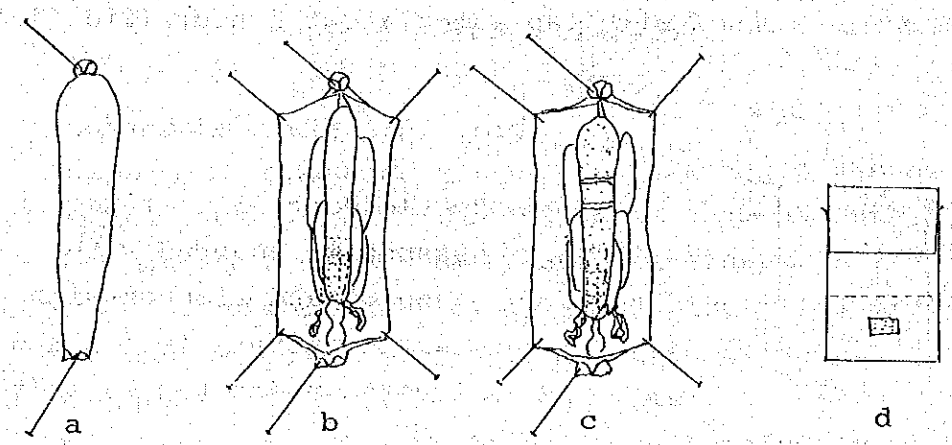

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Fig. 11: Fixation of grown larva.
a: Firm anaesthetized larva on anatomy dish.
b: Open the larva and pour a good amount of fixative.
c: After 20 minutes, dissect tissue and put in fixative containing fixing bottle.
d: Refixation(remained time).

Pupae and moth are treated as same as grown larva. However,

some tissues such as regenerative organ must be removed before fixing. Because they are between fat bodies and it is verry difficult to find out them after fixation. Removed tissue must be put immediately in fixative containing bottle.

3. Treatment after fixation.

a. Rinsing.

Fixed tissue must be rinsed. The tissue must be rinsed after fixation. Tissue fixed with formalin containing solution must be rinsed with tap water about 2 hours(Fig. 12).

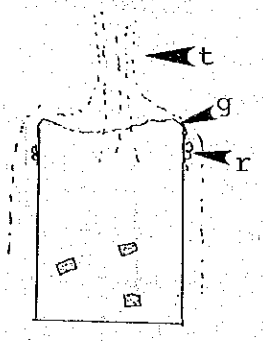

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Fig. 12: Rinse of tissue.

Bottle containing fixed tissues is covered with gauze(g) and stopped with elastic rubber ring(r) and situated under tap water(t).

If fixed tissues must be stocked long time without embedding, gradual ethyl alcohol seriese is prepared and tissues are passed the seriese from low concentration to high ones, and stocked finaly in 80% ethyl alcohol(Fig. 13).

The tissues fixed with Carnoy solution are rinsed three times with 95% ethyl alcohol(30 minutes - one hour for one time of rinsing). If the tissues must be stocked without embedding, they are put in 80% ethyl alcohol and stocked.

Small piece of filter paper writen data may be better accompaning with tissues for marking.

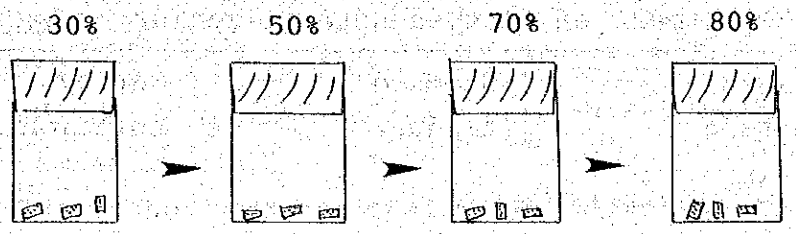

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Fig. 13: Dehydration and stock of fixed tissues.

b. Dehydration.

The tissues fixed with Carnoy solution are rinsed 95% ethyl alcohol and passed three times in absolute ethyl alcohol (15 - 20 minutes for one time), and transfered in ethyl alcohol xylene mixture(1 : 1) about 30 minutes, thereafter they are transfered in pure xylene. Pure xylene is changed three times(15 - 20 minutes for one time).

The tissues fixed with formalin containing solution are also dehydrated and transfered to xylene afetr rinsing with water as mentioned above.

c. Transferation of the tissues to paraffin.

After transferation the tissues to xylene, the tissues are transfered to melted xylene paraffin mixture(1 : 1) about one hour, then they are passed three times in pure melted paraffin(more than one hour for one time).

General schedule of treatment after fixation is summerized as follows.

Table 1. Treatment of tissues after fixation.

Time	Fixative containing formalin	Stocked tissues	Carnoy solution
2 - 3 hours	rinse with tap water		rinse with 95% ethyl alcohol (30 min X 3)
20 min.	30% ethanol		
"	50% "		
"	70% "		
"	80% "	(80%)	
"	95% "	95% ethanol	
"	100% "	100% "	100% ethanol
"	100% "	100% "	100% "
"	100% "	100% "	100% "
30 min.	xylene + ethanol	xylene + ethanol	xylene + ethanol
"	xylene	xylene	xylene
"	"	"	"
one hour	paraffin + xylene	paraffin + xylene	paraffin + xylene
"	paraffin	paraffin	paraffin
"	"	"	"
"	"	"	"

4. Embedding.

a. Materials.

Following materials are required for embedding.

i). Embedding boat.

The author uses generally ceramics boat for making ash for chemical analysis. Boat of hard paper is also available. The paper boat must be made considering amount of paraffin (Fig. 14).

ii). Glyveline.

Glycerine is smeared on inner surface of embedding boat. Unless, it may be very difficult to remove out embedded paraffin cake from the boat.

iii). Pincettes.

Fine pincettes are required for arrangement of tissues on bottom surface of embedding boat.

iv). Alcohol lamp or gass burner.

Pincettes must be kept hot during embedding procedure. Gass burner is better for the purpose. If the burner is not obtainable, alcohol lamp is enough for the purpose.

v). Water bath.

After arrangement of the tissues on bottom surface of embedding boat, it is better to cool quickly paraffin in cold water. A certain quantity of basin(more than 20 cm in diameter) is required for the purpose. In basin, pour cold water stocked in refrigerator. It may be also give the best results using ice blocks.

b. Procedure of embedding.

1). Prepare embedding boat.

2). Smear a drop of glycerine on inner surface of embedding boat.

3). Burn alcohol lamp.

4). Bring parafinized tissues from paraffin oven and pour paraffin with tissues in the boat.

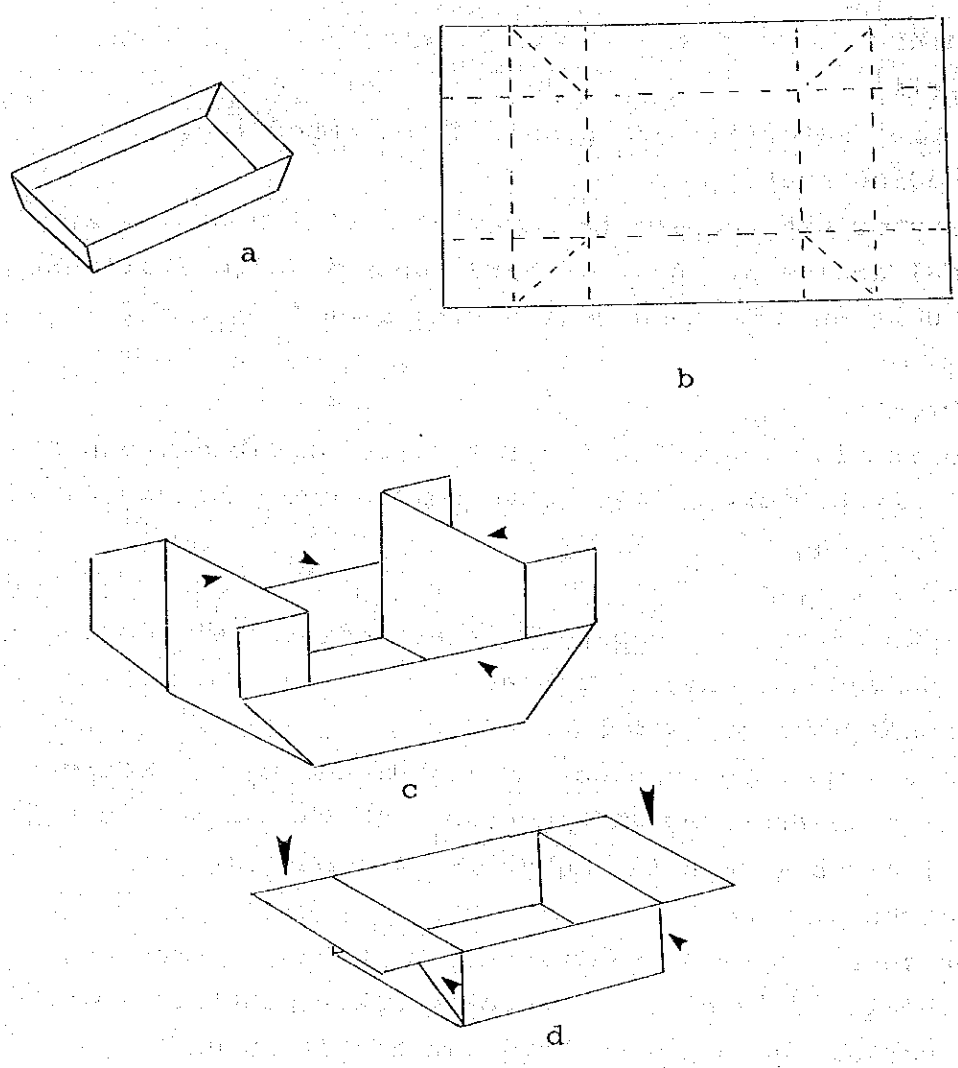

Fig. 14. Embedding boat.

a: Boat of ceramics.

b: Prepare 4 X 7 cm of hard paper and mark folding line.

c: Fold up each side following arrows.

d: Fold down doubled two sides.

5). Arrange the tissues on bottom surface of boat and the label aside of boat, as possible as quick.

6). Cool the boat carefully in cold water and dip whole of boat in water after surface of paraffin cake is harden.

7). Remove paraffin cake from the boat after the cake become so hsrd.

8). Dry the cake under room condition and stock the cake under cool condition.

5. Triming of embedded block.

Embedded tissues are cut off from paraffin cake and trimed as follows(Fig. 15).

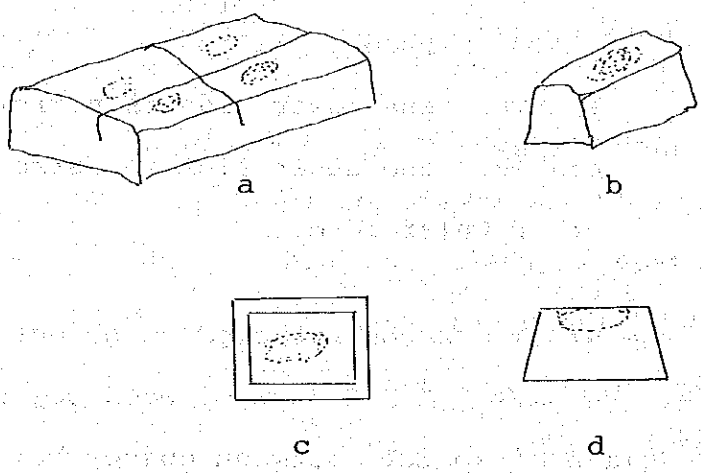

Fig. 15: Triming method of embedded tissues.
a and b: Cut off the block including the tissues from the cake.
c and d: Cut off paraffin around the tissue as opposite edge become parallel.

The block is trimed as surface plane become square or rectangular. Each opposite pair of surface edge must be parallel. If they are not parallel, paraffin ribbon after sectioning dose not become straight(Fig. 16).

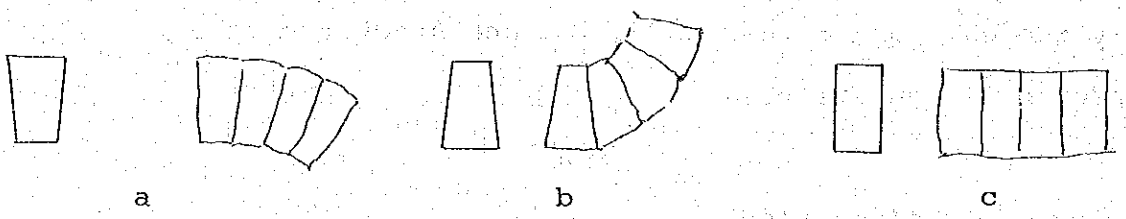

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Fig. 16: Effect of triming to the condition of ribbon.
a: The case lower side is narrow.
b: The case upper side is narrow.
c: Rehular form.

After trimina, the block must be attached on object holder usina heated spatula. Prepare 2 X 2 X 2 cm of wood object holder. Hold heated blade of spatula between object holder and one surface of the block opposite site of the tissue and slide out the blade. Warmed and melted paraffin by blade of spatula fixs the block on the holder after cooling. Label the data on a side of the holder(Fig. 17).

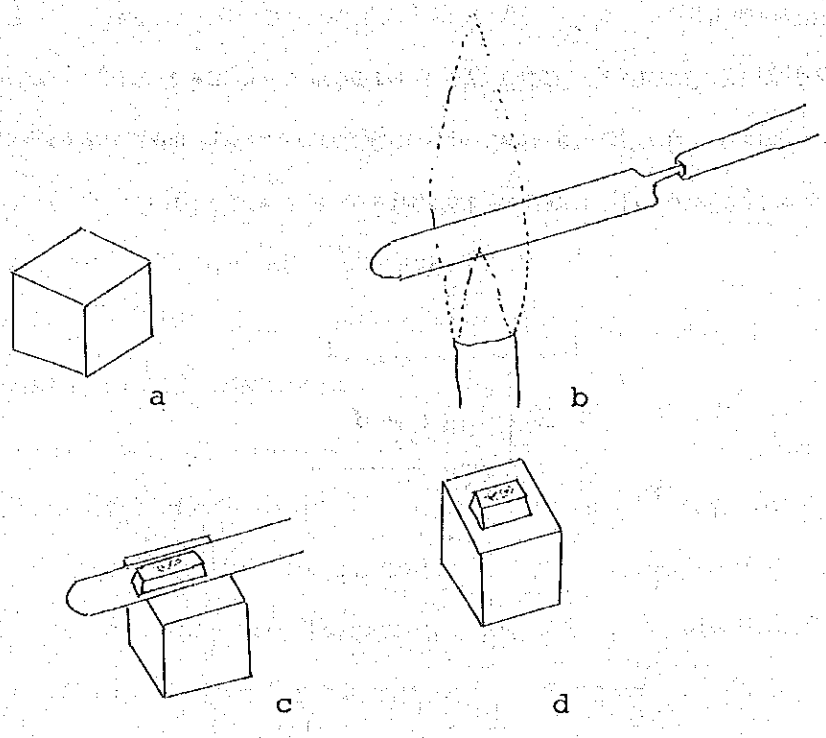

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Fig. 17: Attach the block on the holder.

a: Block holder of wood.

b: Heat blade of spatula.

c: Hold the blade of spatula between block holder and aside of block opposite the tissue, then slide out the blade.

d: Heated and melted paraffin attachs the block firmly on the holder.

6. Sectioning.

Object holder attached paraffin block is inserted in microtome and regulated as the surface to be cut may traverse across blade of nife as in the same plane(Fig. 18).

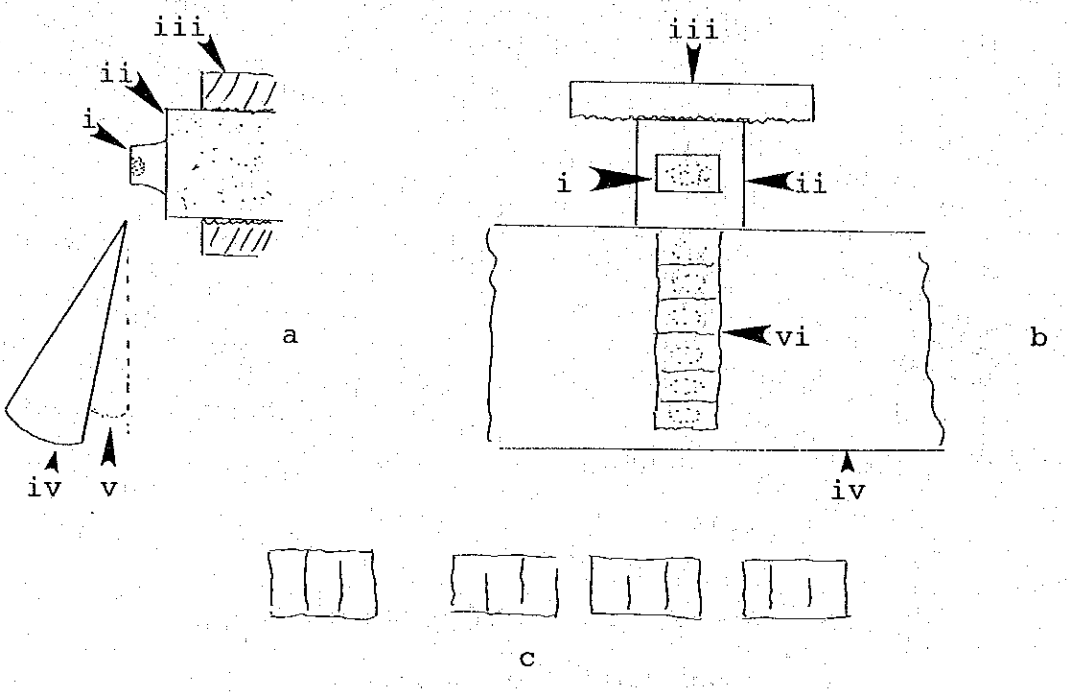

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Fig. 18: Sectioning of the block.

a: Object holder(ii) is inserted into the holder adapter(iii) and firmed. Cutting plane of the block must be regulated to traverse rightly across blade of knife(iv). Clearance angle(v) is also regulated suitably for sectioning.

b: Edge of blade(iv) and parallel edge of the block(i) must be paralleled exactly.

Cut several sections till a section become square or rectangular as previously trimed, and section contain desired part of tissue. Then adjust the microtome to desired thickness. Cut the sections in rapid succession. The sections may form a string as ribbon(Fig. 18, b-vi).

7. Treatment of sections.

a. Dissection of ribbon.

Lift off the ribbon from microtome blade using painting brush needle onto black paper sheated container. Then dissect the ribbon as desired length using fine blade knife such as shaving blade(Fig. 18, c).

b. Sealing of the section.

Well cleened slide glass must be prepared. Put a drop of Glyceline-egg albumin mixture on slide glass, and smear well the albumin on the glass as possible as thinner using fingar. Fingar must be slided quickly and frequently on the glass. After smeared the albumin, put a drop of distiled water on the glass and float dissected paraffin ribbon on water on the glass, and place the glass on hot plate to heat and dry the glass.

Glyceline-egg albumin is made as follows:

Egg albumin	one part
Glyceline	one part
Thymol	one crystal

Mix well the albumin and glyceline and filtrate the mixture using hygine cotton and filter paper. Add a crystal of thymol and keep in well stoppered bottle under cool condition.

c. Deparaffinization.

After sections sealed glass is dryed, the glass is brought to water passing through xylene(two times), absolute ethyl alcohol(two times), 95%, 90%, 80%, 70%, 50% and 30% ethyl alcohol. Time in each treatment is 5 - 10 minutes.

d. Staining and mounting.

Sections transfered to water is stained. Staining methods will be dercribed in following chapter. Stained sections are rinsed and dehydrated through gradual alcohol seriese as mentioned above, and brought to xylene, and mounted. For mounting, diluted Balsum canadacum or Caedax is used. Namely, some staining such as Giemsa and Azan require neutral mounting material as Caedax.

IV. STAINING METHODS.

= Application for the silkworm diseases =

In this chapter, staining methods which are applicable for studies of the silkworm diseases are described, namely Viral diseases and Pebrine. However, these methods are also applicable for Bacteriosis and Mycosis.

1. Hematoxilin-Eosin staining.

a). Kind of hematoxylin dyes.

Staining methods using hematoxylin are applied generally for staining of nucleus of cells. The well known dyes are Delafield's, Ehrich's, Harris', Heidenhain's, Weigert's, etc.

Mayer's hemalum-eosin method mentioned in here is simple and gives sufficient results for studies of the Pebrine and Viral diseases.

b). Mayer's hemalum-eosin staining.

i). Dye solution A.

Hematoxylin	0.1 gr
Sodium iodate	0.02 gr
Pottasium alum	5 gr
Chloral hydrate	5 gr
Citric acid	0.1 gr
Distiled water	100 ml

Dissolve hematoxylin in boiling water. Add Sodium iodate and Pottasium alum after cooling and shake to dissolve. Finally, add Chloral hydrate and Citric acid and dissolve them.

ii). Dye solution B.

Eosin	0.2 gr
95% ethyl alcohol	100 ml

c. Procedure.

1). Bring section to water.

2). Rinse section with distiled water.

3). Dip section in dye solution A and stain within 5 minutes.

4). Rinse section with tap water more than 5 minutes.

5). Dip section in dye solution B and stain 2 - 3 minutes.

6). Rinse section with 95% ethyl alcohol within one minutes.

7). Pass in absolute ethyl alcohol 2 times, 3 - 5 minutes for each time.

8). Pass xylene 2 times, 3 - 5 minutes for each time.

9). Mount with balsam or lesin.

d. Results.

Nucleus of the cells is colored as blue while cytoplasm is colored as pink.

e. Application for Pebrine.

Pebrine protozoa multiply in host cells, in hemocytes, mid gut epitherial cells, muscle cells, silkgland cells, malpighian cells, epidermal cells and regenerative cells. In the host cells, the protozoa show diprokarya form(double nuclei form). The diprokarya form is distinguished as a pair of small blue grains in cytoplasm of the host cells. Spore of

the protozoa may be colored as pink on the surface containing blue grain(sometimes double grains) on a side of inner part.

f. Application for nuclear polyhedrosis.

The method is available for distinguish the nuclear polyhedrosis. Because the disease cause alteration of nucleus of the host cells, hemocytes, fat body cells, silkgland cells, nervous system cells, trachea matrix cells and epidermal cells. Iniyial sign of the disease is aggregation of chromatin in central area of the nucleus; formation of hallo in periphery of chromatin which is called ring zone, thereafter polyhedra are formed in ring zone(Fig. 19). By the way, polyhedra is not

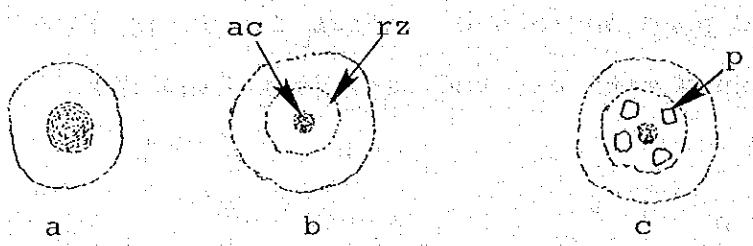

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Fig. 19: Symptom of nuclear polyhedrosis virus infected cell.
a: Normal cell.
b: Accumulation of chromatin(ac) and formation of ring zone(rz).
c: Formation of polyhedra(p) in ring zone.

stainable by both hematoxylin and eosin. Hydrolysis by a certain acid or heating can increase the stainability.

i). Hydrolization by 1 N HCl at 60°C, 5 - 10 minutes.

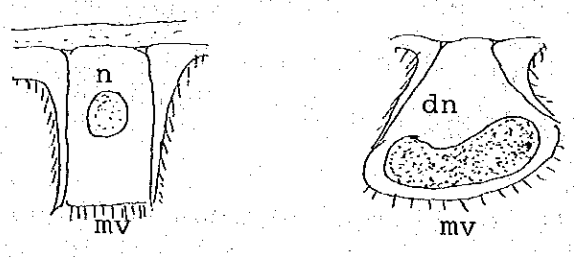
ii). Hydrolysis by 50% acetic acid at room temperature 5 - 10 minutes.

iii). Heating by boiling distiled water in a few minutes.

The polyhedra may be stained by eosin after treatments mentioned above. It may be better to compare two stained preparations, one which is stained without treatment mentioned above, and other which is stained after treatment mentioned above, to distinguish the polyhedra. Hydrolyzation by heated 1 N HCl is recommended for the method.

g. Application for densonucleosis.

The virus infect to midgut epitherial cells of the silkworm. Hypertrophied and densed nucleus of infected cell is able to identify by the method with altered cell form(Fig. 20).



a b

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Fig 20: Alteration of migut epitherial cell by infection of Densonucleosis virus.

a: Normal cell with normal nucleus.

b: Infected cell with densed nucleus(dn) and altered form. mv: microvili.

2. Feulgen reaction.

This reaction is based on composition between aldehide base and sulfided basic fuccin(Schiff's reagent). Aldehide base and Schiff's reagent make a composition showing reddish colorization. For DNA, 1 N HCl is used as catalyzer for hydrolyzation to exposure aldehide base.

a. Reagents.

i). 1 N HCl: One normal hydrochloric acid is heated to 60°C exactly.

ii). Schiff's reagrnt.

Basic fuccin	1 gr
Distiled water	200 ml
1 N HCl	20 ml
Anhydrous sodium bisulfite	1 gr

1). Boil 200 ml distiled water, and add basic fuccin slowly in boiling water. If a large amount of the fuccin is added suddenly in the water, explosive boil may be arised to induce accident.

2). After basic acid is dissolved, cool the solution to 50° C and filtrate the solution using filter paper.

3). Add 20 ml of 1 N HCl and cool to room temperature.

4). Add 1 gr of sodium bisulfite and keep the solution in well stoppered bottle under cool condition.

If reddish color is remained after two days, add few grams of activated chacoal and shake the bottle and filtrate again.

iii). Sulfurous acid solution.

Stock solution A.	10% sodium metabisulfite
Stock solution B.	1 N HCl
Rinsing solution.	
Stock solution A.	6 ml
Stock solution B.	5ml
Distiled water	100 ml

iv). Counterstaining solution.

Light green	1% water solution
Orange green	2% water solution with 1% phospho-tungstic acid

b. Procedure.

1). Bring section to water.
2). Rinse section with distiled water.
3). Hydrolyze section in 1 N HCl heated to 60°C, 5 - 10 minutes.
4). Wash the section with tap water and rinse with distiled water.
5). Dip in schiff's reagent at room temperature, 15 - one hour.
6). Rinse with sulfurous acid solution 3 times, 2 minutes for each time.
7). Wash with tap water about 5 minutes.
8). Rinse with distiled water.
9). Dip in counterstain solution, 3 - 5 minutes.
10). Rince with distiled water about one minute.
11). Rince with 95% ethyl alcohol about one minute.
12). Pass in absolute ethyl alcohol 2 times, 3 minutes for each time.

13). Pass in xylene 2 times, about 3 minutes for each time.

14). Mount.

Note: Time of hydrolyzation must be determined by preliminary examination for fixative and fixing time.

c. Results.

Chromatin in nucleus is colored as red while cytoplasm is stained as green or orange respectively by counterstain solution.

d. Application for Pebrine.

Diprokarya of Pebrine protozoa in cytoplasm of host cell are distinguished by the method as paired red grains. By using orange green for counterstain, the spores are colored as golden yellow containing paired red grains.

e. Application for Nuclear polyhedrosis.

Ring zone is identified clearly by the method. Polyhedra in ring zone are colored by counterstain. By Feulgen reaction alone, peripherry of polyhedra is stained slightly, showing difficulty to distinguish the stainability.

f. Application for Densonucleosis.

Densed nucleus is colored deeply as red by the method.

3. Pyronine Methyl green staining.

This method is used for staining of nucleic acid. DNA is stained by methyl green while RNA is stained by pyronine when the mixture is used for staining.

a. Dye solution.

Both pyronine and methyl green contain another dyes as contaminated ones. Water solution of both dye must be washed as follows:

Pyronine	5 gr
Methyl green	2 gr
Distiled water	100 ml

1). Add same volume of chloroform in dye solution and shake well.

2). After several minutes, chloroform layer may precipitate as bottom layer. Brin upper dye layer and repeat again 2 times.

Washed dye solution is kept as stock solution. The solution is diluted 10 times with 0.1% acetic acid and used for staining.

b. Procedure.

1). Bring section to water.
2). Rinse the section with distiled water.
3). Dip the section in staining mixture solution, 5 - 10 minutes.
4). Blot with filter paper slightly.
5). Rinse with normal butyl alcohol, fer seconds.
6). Pass in tert-butyl alcohol 2 times, 3 - 5 minutes for each time.
7). Pass in tert-butyl alcohol xylene mixture, 3 -5 minutes.
8). Pass in pure xylene 2 times, 3 - 5 minutes for each time.
9). Mount.

c. Results.

Nucleus is colored as blueish green while cytoplasm is colored as pink to red.

d. Application for Pebrine.

Diprokarya of the protozoa is recognizable as green paired grains in cytoplasm of host cells.

e. Application for Infectious flacherie.

The virus infects goblet cells in midgut epitherium. Attacked cells degenerated, and some degenerated ones are included in cylinder cells. Degenerated goblet cells are colored deeply as red.

f. Application for Densonucleosis.

Densed nucleus is colored deeply as green.

g. Application for Nuclear polyhedrosis.

Ring zone is distinguished by the method.

4. Hamm's Azan stain.

The method was developed for Polyhedrosis and Granulosis. The method is also applicable for Pebrine showing beautiful coloration of tissues.

a. Dye solutions and rinsing solution.

i). Dye solution A.

Azocarmine	2 gr
Distiled water	100 ml
Acetic acid	1 ml

Boil distiled water and add azocarmine to dissolve. Cool to 50°C and add acetic acid. Filtrate after cool to room temperature.

ii). Dye solution B.

Orange green	2 gr
Fast green(SCF)	1 gr
Aniline blue	1 gr
Phosphotungustic acid	1 gr
Distiled water	100 ml

iii). Aniline water.

Stock solution	
Aniline	10 ml
95% ethyl alcohol	90 ml
Rinsing solution	
Stock solution	10 ml
Distiled water	90 ml

b. Procedure.

1). Bring section to water.
2). Rinse section with distiled water.
3). Dip in 50% acetic acid, 5 minutes.
4). Rince with 1% acetic acid.
5). Dip in dye solution A, 15 minutes.
6). Rinse with distiled water.
7). Rinse with aniline water, 10 seconds.
8). Rinse with distiled water 3 times, 10 second for each time.

9). Dip in dye solution B, 15 minutes.

10). Rinse with 70% ethyl alcohol, 30 minutes.

11). Pass in absolute ethyl alcohol 3 times, one minutes for each time.

12). Pass in pure xylene 2 times, 3 - 5 minutes for each time.

13). Mount with neutral mounting lesin.

c. Results.

Many tissues are differenciated as various colors as follows:

Epidermis: epicuticle-orange, exocuticle-red, endocuticle-blue.

Silkgland: cell layer-blue with red nuclei, sericin layer-red to orange, fibroin layer-blue to green.

Muscle: red, purple to orange.

Nurvous cord: blue to purple.

Malpighian tube: blue

Fat body: blue to orange.

Connective sheath: blue to green.

d. Application for Pebrine.

Schizonts are colored as brownish gray to graish orange with reddish paired grains of diprokarya. Spores are colored as golden yellow with reddish paired grains of diprokarya.

e. Application for polyhedrosis.

Polyhedra are colored red to orange, occasionaly blue to green.

V. MICROPHOTOGRAPH OF SILKWORM DISEASES

1. Protozoasis.

a. Leptomonas bombysis.

The infection causes by feeding of the cysts. Thereafter emarged promastigotes from the cysts attach themselves on microvili of midgut epitherium. There is no clear effect by the infection during larval stage of the silkworm. However, promastigotes invade in hemocoel after pupation and they multiply in hemocoel(Fig. 21-a). Then they form the cyst(Fig. 21-b). The pupae can not eclose to imago. The encystation is also observable during larval stage inlumen and the cysts are excreted from lumen with soils and become new infectious source.

b. Nosema spp.

The infection causes by feeding of the spores. Thereafter they multiply in various tissues(Fig. 22) showing schizogonoc and sporogonic stages. The infection causes also from mother moth to her eggs(transovium transmission). Transovium transmission occurs at pupal stage.

c. Pleistophora sp.

The infection causes by feeding of the spores. The protozoan multiplies only in cylinder cells of midgut epitherium showing multiple fission(Fig. 23) in membrane of pansporoblast.

2. Cytoplasmic polyhedrosis.

The diseases is one of injurious viral disease in South Sulawesi State. Infection causes by feeding of polyhedral bodies. Virus particles are released from the inclusion body after enter in midgut and free particles invade maunly in cylinder cells of midgut epitherium. Thereafter they multiply and form polyhedral bodies in the cytoplasm. Polyhedral bodies are ocasionaly formed in nucleus. The material obtained in the State

has various form of polyhedra, cube in nucleus, cube(Fig. 24, B-s), dodecahedron with cube(Fig. 24, C-o), and rombic dodecahedron(Fig. 24, D-r) in the cytoplasm.

3. Nuclear polyhedrosis.

Infection causes by similar mechanism of Cytoplasmic polyhedrosis. But the virus multilies in various tissue cells, blood cells, silkgland cells, fat boy cells, epidermal cells, etc. Initiation of infection is recognizable by formation of ring zone in nucleus of host cells. Chromatin is accumulated in center area of nucleus and halo is formed between nucleic membrane and accumulated chromatin(Fig. 25, A). Thereafter polyhedral inclusion bodies are formed in ring zone(Fig. 25, B).

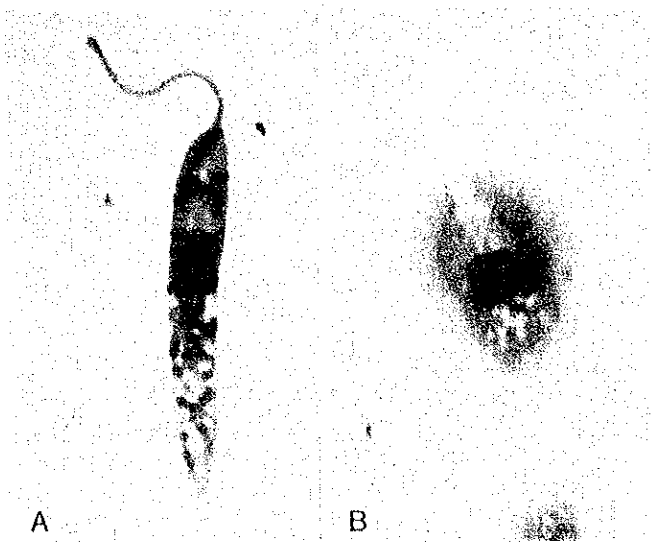

Fig. 21: Leptomonas bombycis (X 4,000).
A: Promastigotes in multiplication.
B: Encystment.

Fig. 22: Nosema bombycis.
A: Spores multiplied in muscle layer (arrow).
B: Sporoblast(sb) and spores(sp).

Fig. 23: Pleistophora sp. in midgut epitherial cell. Arrows show pansporoblast under multiple fission.

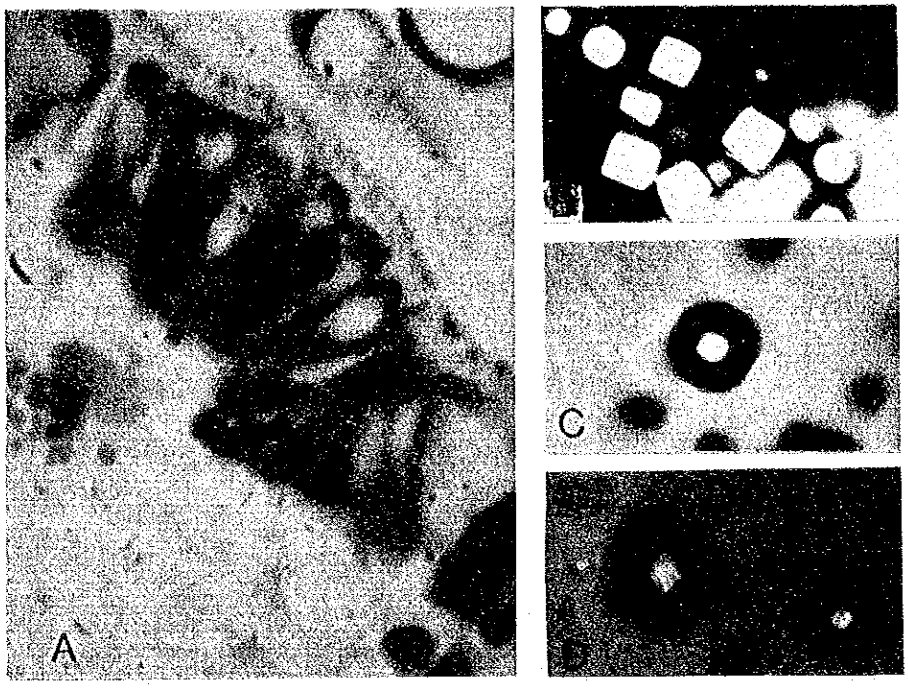

Fig. 24: Cytoplasmic polyhedrosis.
A: Midgut epitherium infected by cytoplasmic polyhedrosis virus.
B: Cubuc form of polyhedra.
C: Dodecahedron with cube.
D: Rombic dodecahedron.

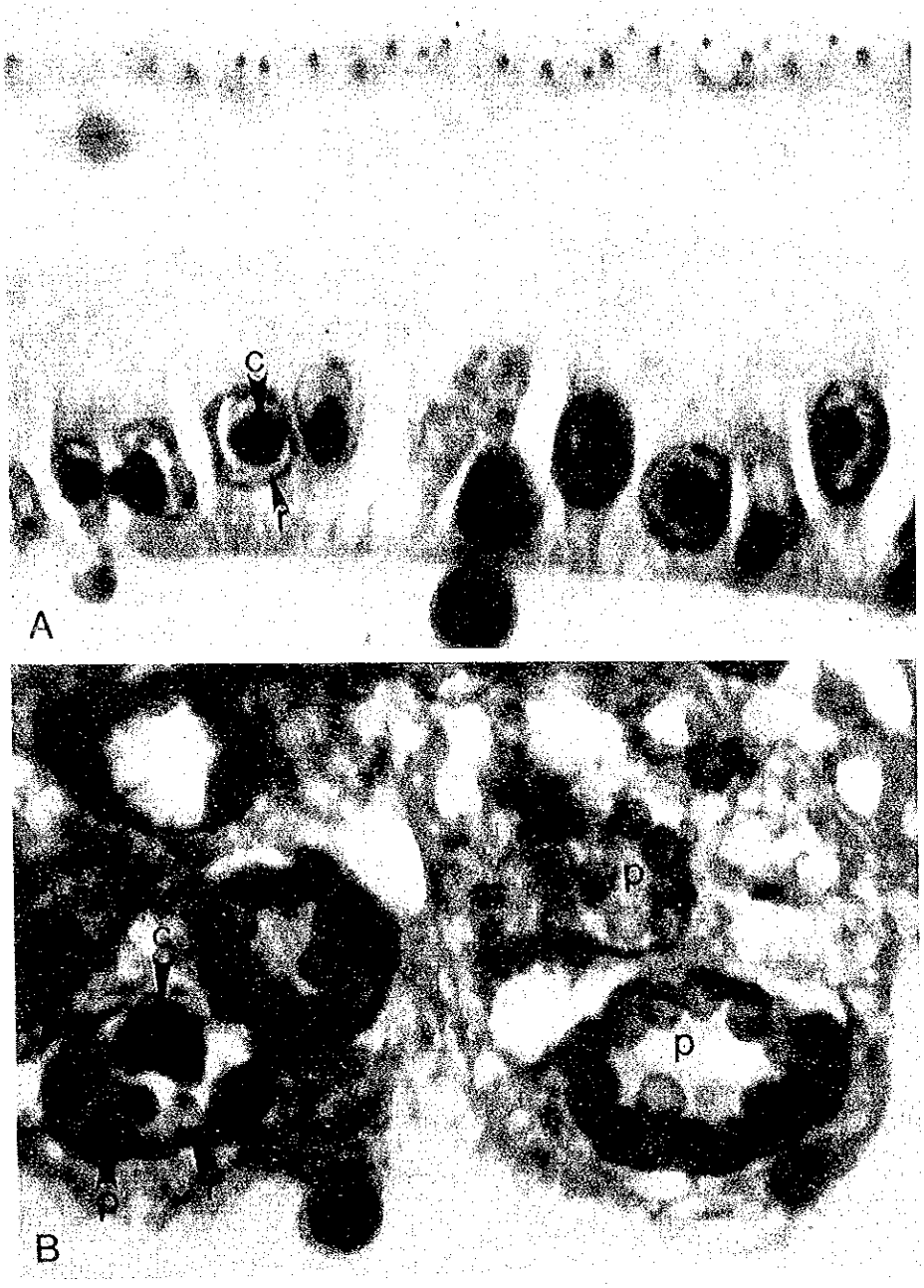

Fig. 25: Nuclear polyhedrosis.
A: Epidermis infected by the virus Showing chromatin mass(c) and ring zone(r).
B: Fat body infected by the vitus showing numerous polyhedra(p).

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G

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APPENDIX

MONOGRAPHS OF TECHNIQUE

MacManus, J. and Mowry R. Staining Methods. Harper and Row, Evanston and London, 1960.

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PART Ⅱ

CONTROL METHOD OF PEST INSECTS IN MULBERRY FIELD

BY

YOSHIHIKO ABE

INTRODUCTION

The most injurious damages on mulberry field in South Sulawesi State are caused by insects. Since begining of the activities of Sericulture Development Cooperation Project, we have been investigating real conditions of injures by the pest insects, and four key pest insects have been made clear out at present. Thereafter a part of life cycle of these key pests have been made clear out, and control methods of the pest insects have been established. In this process, we have noticed that some artificial factors have been also inducing outbreak of the pest insects as well as natural factors. Alteration of rainy season and dry season have been considered to be a factor having the greatest influence on the outbreak. On the other hand, it is clear that lacking of consideration on air circulation arround and in mulberry field induces serious damages by mulberry white scale, imcomplate triming of mulberry stumps keeps the pest insects on the stumps to emphasize damages on newly pushed shoots. In spite of existence of pyralid who preferes to attack on high trained stumps, medium and high cut training is done to emphasize the outbreak. It is also clear that thses artificial factors are now inducing serious damages by pest insects in the State. In another word, these artificial factors emphasize the insects to become injurious one for mulberry fields. These are typical examples that common insects become harmful ones for agriculture. It is also observable that outbreak of a certain pest insect induces infestation of another pest insects or diseases on mulberry trees showing that damaged stumps are destroied at heels by these pest insects

We have systematized control method of these pest insects with regarding agronomic countermeasures following investigation of seasonal prevalence and factors inducing the outbreak. However, it was very difficult to make spray calender regarding seasonal prevalence of the pest insects. Because the seasonal prevalence is influenced by regional and meteorological factors.

Meteorological factors are variable inthe State. To say exactly, we have no clear data concerned on seasonal prevalemce, and the data must be gathered and analized by long term observations.

By the way, mulberry shoots are harvested constantly in a certain period. Training and triming must be done at this time exactly, and it is easy to take countermeasures what we extracted on this paper for control of the pest insects at this time. We believe that our methods mentioned here give sufficient effects for control the pest insects.

We have recomended four insecticides regarding our marketing researchs in the State, screening tests, verifying tests and japanese data. The screening must be followed to find out good insecticides adaptable for moriculture. However, insecticides are not so superexcellent for control the pest insects without combining with agronomic countermeasures. Therefore, we must claim that the agronomic countermeasures are basic methods for control the pest insects, and application of insecticides is only supporting method of the agronomic countermeasures. Following descriptions are writen basing the idea mentioned above.

I. MEALY BUGS

A. Life history and habit.

Main harmful mealy bug species on mulberry tree is identified as Maconellicoccus hirustus GREEN(Hibiscus mealy bug). However, there are also some unifentified species on mulberry tree, similar to Pseudococcus adnium or P. comstocki. They can observe through a year on mulberry tree. Serious outbreak begins at the end of rainy season and terminates bigining of rainy season. However serious outbreak is observed through a year in the regions where dry season and rainy season do not alter clearly. Bigining of rainy season, some species of lady bird beetle and larva of Chrysopha sp. are observable as praydetors. These praydetors seem to have a role for decreasement of population of mealy bugs.

Egg stage is about five days. Larval atage is about 25 days showing three instars, therafter they become imago. Total life duration is about 35 days. Eggs are layed on base of petiole or hind of rolled leaf(Fig. 1, B).

B. Damages.

The larvae parasite on terminal bud, namely base of petiole and suck juice of tree. Parasited terminal bud is dwarfed showing nod(Fig. 1, A) and dwarfed buds and leaves become good habitat for mealy bugs, namely, dwarf shoots become center of dispersal of mealy bugs if the shoots are remained on trimed stumps(Fig. 1, C). Dwarf of terminal bud gives damages for young silkworm rearing. Damages of terminal bud induces sprouting of lateral bud and early leaf fall.

C. Conditions inducing the outbreak.

As was mentioned above, mal triming without elimination of dwarf shoots is one of inducing factor of serious outbreak of mealy bugs. These pest insects are common parasitic

insects on plants arround of mulberry field. We have observed two species of Convolvuaceae which are crawling on shoots of mulberry trees(Fig. 2, B), and a species of unkown weed in the field. Kapok(Ceibea pentandra), two species of Legiminoseae, Hybiscus rosa-sinensis and Citrus sp. are parasited by mealy bugs in arround the mulberry field. Some miss management of mulberry field can be suggested in the field where outbreak is serious, i.e., mulberry field is not separated enough from foresty and bush area, weed control is not done, triming is not good, etc.

D. Control methods.

a. Agronomic countermeasures.

To control the mealy bugs, some agronomic countermeasures are effective. Namely, field managements are important for for control the mealy bugs.

a-1. Outerspace arround the mulberry field.

To prevent the invasion of mealy bugs from arround of foresty and bush area to mulberry field, outerspace must be set arround the field. The mealy bugs have possibility to imigrate from bush area to the field. However, our experiment and observation on imigration ability showed that they could not imigrate traversing naked land. Setting of outerspace arround the field may be effective to prevent imigration of mealy bugs from outer area to the field. The detail of setting of the outerspace is described in Fig. 7.

a-2. Training and triming.

Triming must be done carefully and skilfully. Namely, dwarf shoots(Fig. 1, C) become center of dispersal of the mealy bugs to newly pushed shoots. Dwarf shoots must be eliminated completely as is shown in Fig. 8,B. When the leaves are

remained on the stumps after triming, the remained leaves become source of dispersal of the mealy bugs, because egg clasters of mealy bugs are observed frequently observed on hind of these leaves.

a-3. Cutting back.

Cutting back is the best agronomic method for control of the mealy bugs. the method is cutt off of young shoots from base of the shoot to eliminate dormant buds and to induce sprout of latent buds. Our experiments showed that damages of newly pushed shoots by mealy bugs were decreased more than 80% by the method.

a-4. Cleaning of mulberry field.

Weeds in inter ridge and inter stump must be controled. Weed in outer space arround the field must also be controled. Mulberry waste shoots after harvesting and triming are collected and accumulated in outer space for decomposition. These accumulation may be available as a fertilizer in future.

b. Chemical control.

When serious damages is expected, spray Diazinon(X 2,000-3,000), Dibrom(X 1,000) or Dimetoate(X 1,000) immediately after triming. When damages are observed after sprouting the buds, spray the chemicals mentioned above regarding direction of safety use in chapter V.

However, if agronomic countermeasures mentioned above are never taken, effect of these chemicals for control of the mealy bugs is decreased and we cannot prevent extraordinaly damages of mulberry shoots by the mealy bugs.

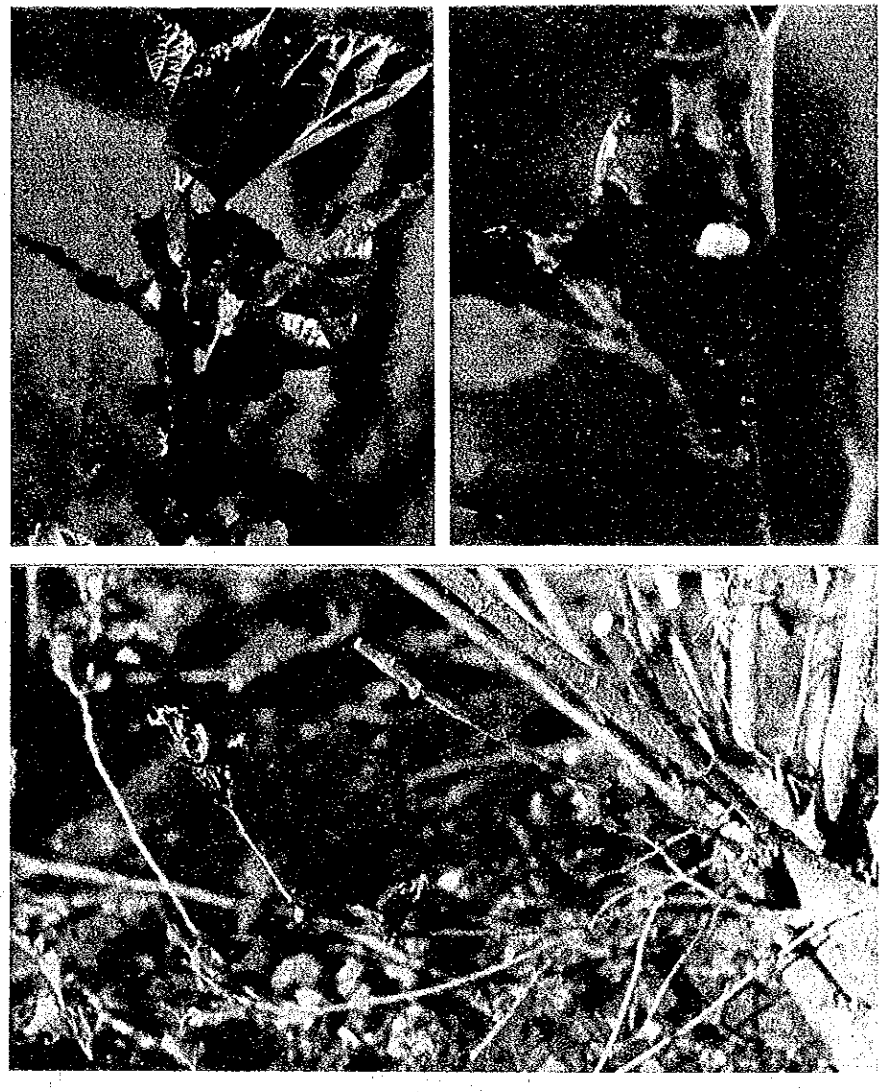


Fig. 1. A: Damages of mulberry shoot by a mealy bug.
B: Egg claster on base of petiole(arrow).
C: Dwarf shoots remained on stumps after triming.

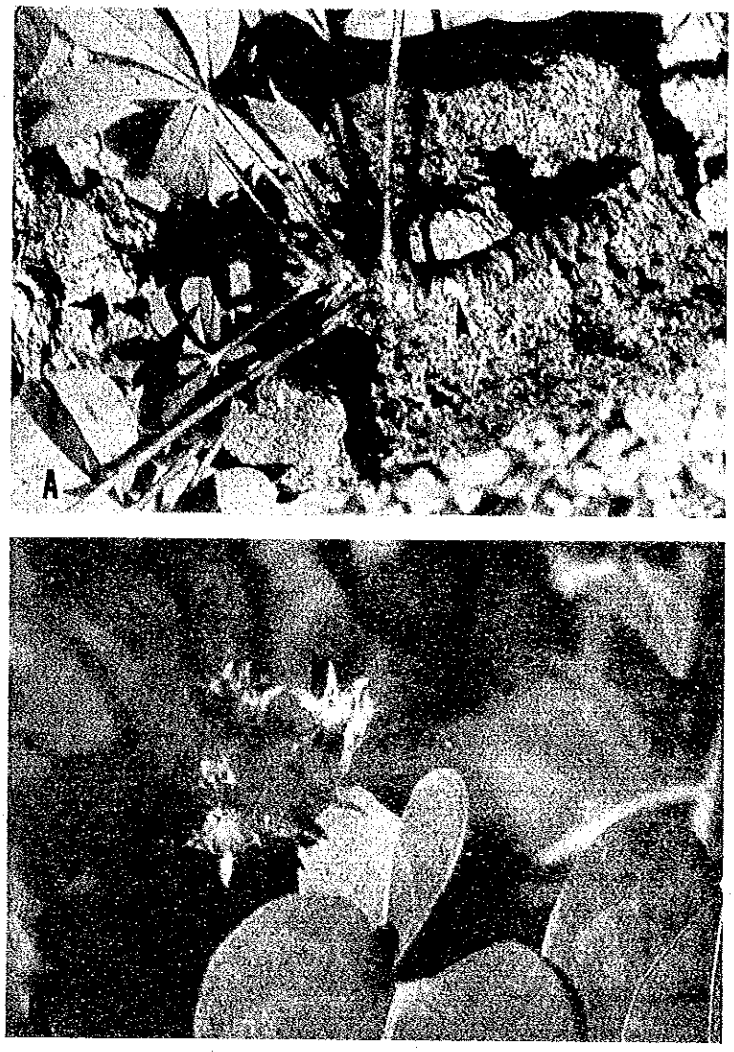


Fig. 2. A: Mealy bugs on terminal bud of Kapok(arrows).
B: Mealy bugs on a species of Convolvulaceae (arrows).

Fig. 3. A: Hibiscus rosa-sinensis parasited by a mealy bug, showing dwarf leaves.
B: Citrus sp., parasited by a mealy bug(arrow).

II. PYRALID

A. Life history and habitat.

The pest insect was identified as Glyphodes pulverulentalis HAMPSON. Its body color is darker than that of mulberry pyralid, showing resemble patchs on the body. Matured larva is about 20 mm in body length, showing numerous black spots on ventral view. Body color is darker than that of mulberry pyralid. Namely, black head is different from mulberry pyralid. The outbreak is observed from end of rainy season to end of dry season seriously. Seven instars are counted during their larval stage, in about three weeks. Pupation is taken in hind of holded leaves on mulberry shoot or hind of leaves on the surface of soil. Eggs are deposited in robe of terminal buds choosing the highest shoots.

B. Damages.

The larvae eat leaves of terminal bud and give damages for grown silkworm rearing. Damages of growing point of the shoots by the larvae induce sprouting of lateral buds and leaf fall in early time and give also damages for grown silkworm rearing. High cut training is very dangerous because the pest insect choose the highest shoots rather than lowest shoots for egg deposition.

C. Conditions inducing the outbreak.

As mentioned above, if the stumps are trained as high cut method, damages by the pest insect become extraordinaly. Enough grown shoots are easily infested because female moth preferes high shoots for egg deposition. Mal triming also induces egg deposition. If the leaves and dwarf shoots are remained on the stumps after triming, the outbreak occurs in early time after triming.

D. Control methods.

a. Agronomic countermeasures.

Our investigations and experiments indicated that the outbreak is also related to field management as well as mealy bugs and mulberry white scale. Therefore, farmers must do carefully general management of the field. Namely, following countermeasures must be done carefully to prevent the outbreak.

a-1. Low cut training and semi-low cut training.

As previously described, the pest insect preferes the highest terminal buds for egg deposition. To prevent the outbreak, stumps must be controled as possible as lower. From entomological aspects, low cut training(height of stump is less than 30 cm) or semi-low cut training(height of stump is 30 - 50 cm) is able to recomend. Namely, low cut training may be better for mulberry trees for grown silkworm rearing.

a-2. Cutting back.

As well as for control of mealy bugs, cutting back is one of the most effective method for control the pyralid. Our experiments showed that damaged shoots were decreased about 60% by the method alone. However, application of insecticides without doing cutting back decreased only 30% of damaged shoots. In contract, application of insecticides after cutting back decreased more than 80% of damaged shoots (Fig. 4).

b. Chemical control.

Diazinon(X 2,000 - 3,000), Dimetoate(X 1,000) and Dibrom(X 1,000) are effective for control the pyralid. When the outbreak is observed spray these chemicals regarding direction of safety use in chapter V. However, these chemicals are not so effective without treatment as mentioned in D-a. Seasonal prevalence of the mealy bugs and the pyralid is very similar. Therefore, if outbreak of both kind of pest insect is expected, spray of the chemical must be done two

times; firstly, just after triming, secondary, 2 weeks after triming.

Fig. 4. A: Mulberry stump without cutting back showing poor growth of shoots after damaged by the pyralid.
B: Mulberry stump after cutting back showing sufficient growth of the shoots.

III. MULBERRY WHITE SCALE

A. Life history and habitat.

A scale insect about 2 mm in body length parasite on mulberry shoots, sucking juice of the trees. Serious outbreak is observed mainly in rainy season. Initiation of serious outbreak appears as white fiber like substances on mulberry shoots(Fig. 5-A). The white substances are secretions by male larvae. Female shows orange scale at this time. Female become pupa after three instars larval stage, thereafter imago ecloses several days after pupation. Imago deposites the eggs and die in a few days after eclosion. Total life duration is considered about 40 days. The pest insect is also observed arround the mulberry field, on Capsicum sp., and another Solanaceae plants, and Papaia.

B. Damages.

Damages by the white scale are various. When the field management is done carefully and skilfully, damages do not appear so serious. However, when the field is treated under miss management, extraordinary outbreak may be induced as in Fig. 5-B, and shoots willdie in future. Unless parasited shoots do not die by parasitism of the pest insect alone, brown lepra or plaster attacks the shoots as a secondary invader on white scale parasited trees(Fig. 5-C) and vitality of shoot is decreased gradualy by secondary invaders. Finally, a kind of longicorn attack on a shoot and gives serious damages to die.

C. Conditions inducing the outbreak.

The white scale outbreak seriously in rainy season. The most serious damages are given to the field under shadowy and humid condition. Fig. 6-A shows the field where shoots are remained without harvesting after grown enough, and grown shoots make shadowy and humid condition showing infestation

of the white scale on stumps. In such a case, the pest insect disperses rapidly in the field. When the shoots must be remained on the stumps for a long time for nursing stumps to obtain vitality, poorly grown shoots and dwarf shoots must be eliminated to obtain good lighting and air circulation; thining of the shoots considering utilizable shoots in future. Fig. 6-B shows typical field under shadowy and humid condition; periferry of the field is covered by the highest plants and cuttings are growing poorly under shadow of the highest plants. Most of cutting are parasited by the white scale to die, and field is replanting. However, cuttings can not grow enough at present, and parasitism of the white scale is observable on newly replanted ones. In both two example, outbreak is occured without relating rainy season, and farmers can obtain only poor vitality stumps, inducing the parasitism of the longicorn beetle following parasitism of brown lepra or plaster. Medium and high cut training are also inducing parasitism of the white scale as well as pyralid, brown lepra or plaster and longicorn beetle.

D. Control methods.

a. Agronomic countermeasures.

a-1. Arrangement of field circumastance.

To prevent the outbreak and dispersal, maintenance of air circulation arround and in the field is necessary. To obtain air circulation, outer space arround the field must be set as in Fig. 7. The space can separate the field from forsty and bush area and prevent shadows on mulberry stumps from the highest trees arround the field. Density woods arround the field are also thined to obtain air circulation. The outer space must be kept cleen, especially weeds must be controled.

a-2. Width of planting space.

When planting space is so narrow, maintenance of air circulation arround the stumps become very difficult after the shoots grow. The interspace between each stump and ridge must be taken as possible as wide(more than 0.5 X 1 m). Weed control arround the stumps is also necessary to maintain air circulation.

a-3. Training.

One of good agronomic countermeasure to prevent serious outbreak of the white scale is low cut training. The method is also effective to prevent pyralid, brown lepra, plaster and longicorn beetle.

B. Chemical control.

To control the white scale using chemicals, rapid treatment is required. If initiation of outbreak is observed as is shown in Fig. 5-A, the shoots must be cut off immediately. We recommend low cut training. Thereafter insecticides as previously described must be sprayed. However, if the prasitism is progressed seriously as is shown in Fig. 5-B and C, more effective countermeasures must be done. After low cut training of all stumps, spray lime sulfur(CaS_x, X 8) immediately. The chemical is also effective for brown lepra and plaster which parasite as secondary invader after parasitism of the white scale. Regarding the agronomic countermeasures, the field must be kept cleen before spraying the chemical, and pest parasited shoots and branchs must be eliminated from the field and burnt out or accumulated in outer space to make compost.

Fig. 5. A: Initiation of outbreak of the white scale. White fiber like substances appears on surface of mulberry stumps and shoots.
B: Serious outbreak showing that the shoot become completely white.
C: Invasion of brown lepra after parasitism of the white scale.

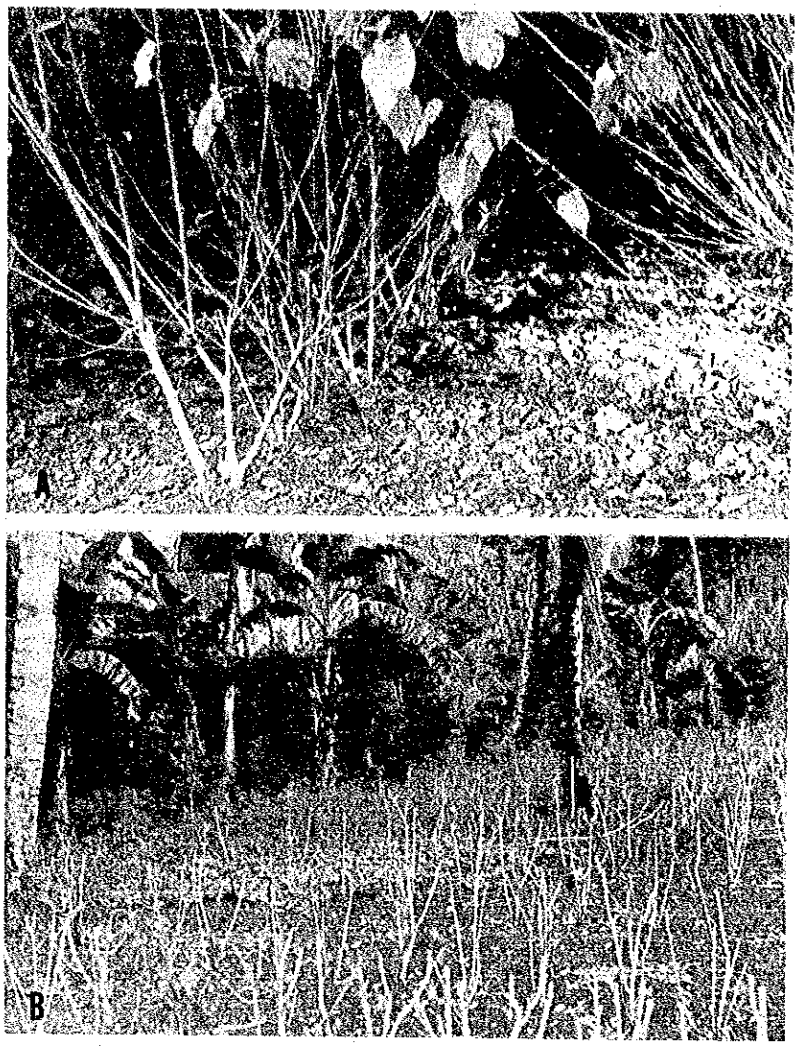


Fig. 6. Conditions inducing serious outbreak of the white scale.

A: Enough grown shoots are remained in the field without harvesting. In the field, the white scale multiplies and disperses rapidly.

B: The field under typically shadowy and humid condition. There is no space separating between the field and bush area, and the white scale multiplies in peripherry of the field and disperse to inner of the field. Replanted cutting can not grow enough showing poor vitality because of the parasitism.

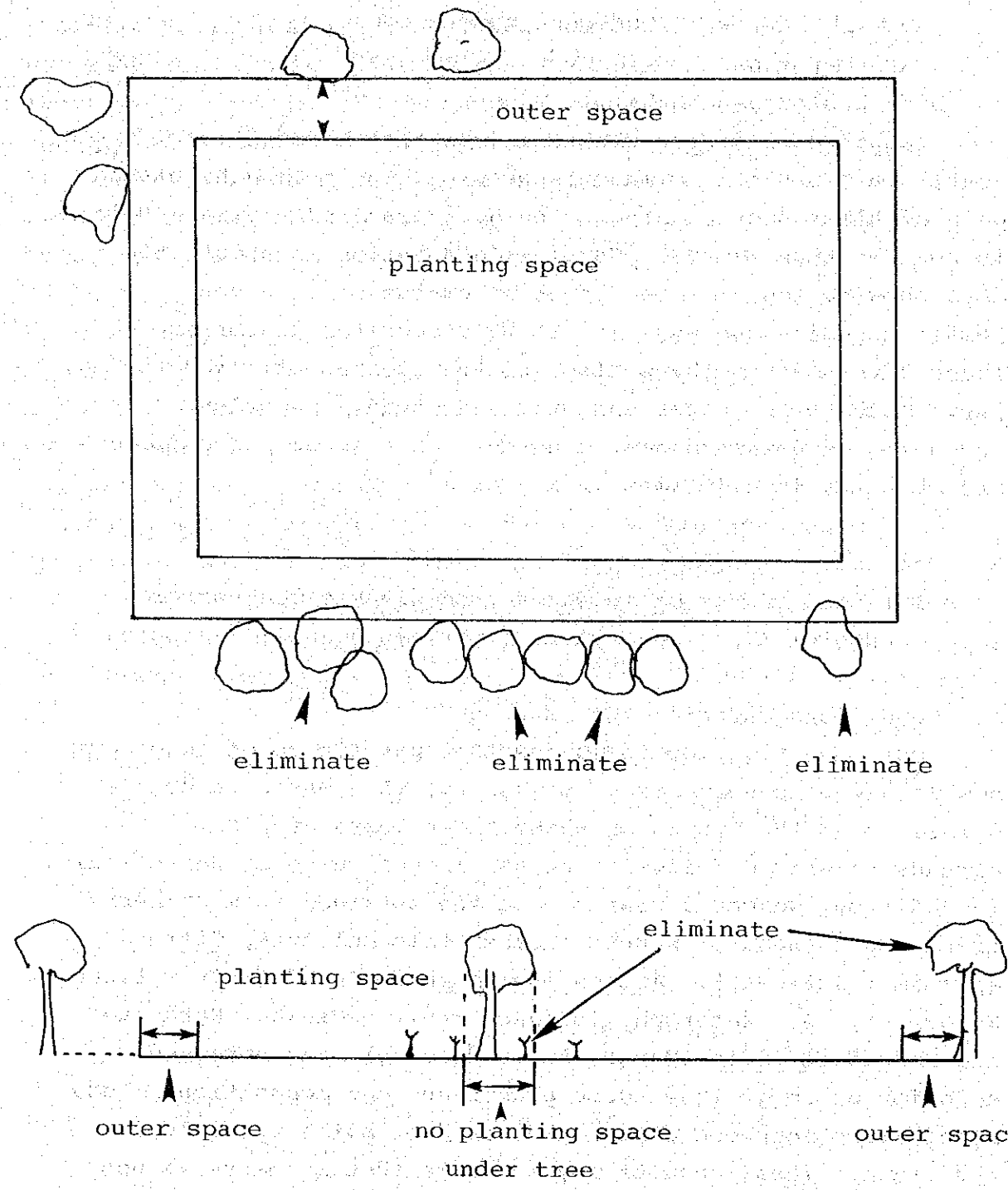


Fig. 7. Lay out of mulberry field for establishment and improvement. Outer space must be set arround the planting space. Density woods are also thinned to obtain air circulation. Plantation under high trees must be avoided.

IV. LONGICORN BEETLE

A. Life history and habitat.

Epepeotes plarator NEWMAN is the most harmful longicorn beetle in the State. Imago is about 20 mm in length, having pair of black spot on hind of head, thorax and elytra. Male is smaller than female. Total life duration is about 160 days showing two or three times of emargence in a year. Female deposits the eggs inserting ovipositor in cortex layer after biting the surface of the shoots. After hatching, larva eats cortex layer and gradualy invades in xylene layer, decending to downward near the root. Emargence and oviposition are observed in all times in a year.

B. Damages.

Infested trunks of mulberry tree die because cortex layer, cambium layer and xylene layer are heavily destroied.

C. Conditions inducing the outbreak.

Our investigation indicates that parasitism of longicorn beetle alone is very rare. Generally, the longicorn beetle parasites on the trunks on which brown lepra or plaster has already parasited following parasitism of mulberry white scale. In addition, we could rear the larvae obtained from mulberry trunks on branchs of plants another than mulberry, Citrus sp.(Lime, Rutaceae), Artocarpus communis(Bread fruit = Sukun, Moraceae), A. Heterophyllus(Jack fruit = Nangka, Moraceae) and Mangifera indica(Mango, Anacardiaceae), and obtained eclosion of imago from these plants and egg deposition. These plant are observable commonly arround of mulberry field in the State. These results may indicate that mulberry is not

specific host plant of the longicorn beetle and pest insect has wide host range. Namely, the pest insect could persist their life cycle in dead branchs of the plants mentioned above, indicating that the pest insect had saprophagy nature. It is also clear that the longicorn beetle is not so harmful pest insect to mulberry tree unless the host is affected by mulberry white scale and brown lepra or plaster.

D. Control methods.

To prevent damages by the longicorn beetle, mulberry white scale must be controled. Because, control of mulberry white scale can prevent infestation of longicorn beetle following infestation of brown lepra or plaster. We recommend low cut training method as a effective countermeasure for control of these related pests.

When the trunks are infested by the pest insect, the trunks must be cut off and burnt out. Because larvae of the pest insect can persist their life cycle in cut-off trunks.

V. GENERAL METHOD FOR CONTROL OF MULBERRY PEST INSECTS.

A. Agronomic Countermeasures.

As described in elswhere in above, it is clear that training and triming methods are very important for control the pest insects. We can recommend that low cut training method is the best for control the pest insects. Thereafter triming must be done completely as is shown in Fig. 8-B. Our experiments showed that cutting back method was the most effective method for control the pest insects(Fig. 4-B, Fig. 8-C). When these methods are done as pretreatment of the stumps for application of insecticides, control effects may be increased sufficiently.

B. Recommendation of insecticides.

We wish to claim again that insecticides are only supporting materials of field management. Following insecticides are obtainable in the State and applicable for mulberry field under direction of safety use. The chemicals are harmful not only to the pests but also to the silkworm and human. Direction of safety use must be kept exactly.

Table 1. Insecticides applicable for control of mulberry pest insects

CHEMICALS	INSECTS	CONCENTRATION (dilution)	RESIDUAL TOXITY (safety days after application)
Diazinon	Mealy bugs Pyralid	X 2,000 - 3,000.	15 days
Dibrom	Mealy bug Pyralid	X 1,000.	5 days
Dimetoate	Mealy bug Pyralid	X 1,000.	7 days
Lime sulfur (CaS_x)	Mulberry white Pyralid	X 8	15 days: Spray just after triming

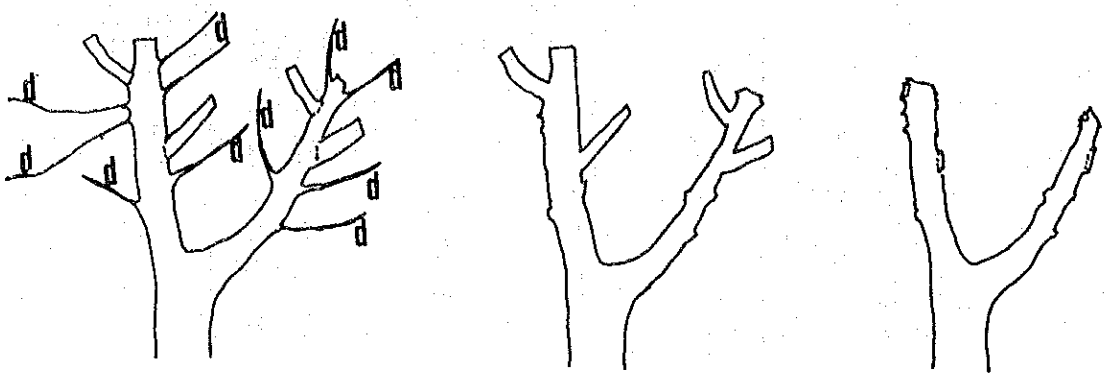


Fig. 8. Triming method of mulberry stumps.

A: A triming method observed generally in the State. Dwarf shoots(d) are remained on the stump on which we can observe larvae and claster of eggs of mealy bugs. New shoots are easily damaged immediately after sprouting from bud. The method also induces egg deposition of pyralid moth on newly pushed shoots.

B: Well trimed stump. Dwarf shoots are completely eliminated.

C: Cutting back. Dead part of trunk is eliminated completely, thereafter the shoots containing dormant buds are eliminated to induce sprouting of latent buds.

Application of insecticides on A is not effective for control the mealy bugs and pyralid. Application of insecticides on B is rather effective than on A. Application on C is the most effective.

B. 桑害虫防除専門家報告書（英文）

THE REPORT OF JAPANESE EXPERT FOR MULBERRY INSECT PEST CONTROL IN SOUTH SULAWESI (ENGLISH)

菊　地　　実

MINORU KIKUCHI

派 遣 期 間

昭和 56 年 9 月 2 日～

昭和 56 年 12 月 1 日

2 SEPTEMBER 1981 – 1 DECEMBER 1981

REPORT ON MULBERRY INSECT PEST CONTROL IN SOUTH SULAWESI

M i n o r u K I K U C H I

Short term Expert

(2 September 1981 - 1 December 1981)

P R E F A C E

This report was realized in Japanese by Mr. Minoru KIKUCHI, Short term Export for control of mulberry pests. The report contains a lot of avairable suggestions for control of mulberry pest insects in South Sulawesi State. He has already reported some opinions extracted from his works in the State. However, the translater considered that his extracted opinions was not enough for progressing sericulture in the State. The translater hopes that all relatives in Sericulture notice on the report and progress the development of Sericulture in the State.

Yoshihiko ABE
Expert of Pest and Disease control

I. PURPOSE

The auther charged Short Term Expert of mulberry pest insect control by order of Sericulture Development Cooperation Project in South Sulawesi State to develop control method of main insect pest in mulberry field in the State during 2 September 1981 - 1 December 1981.

II. RESULTS OF STUDY

The real condition of outbreak of mulberry pest insect in the State was previously informed by former and present Expert of Pest and Disease of the Project. Four main moriculture regions were observed to make clear cut key pests in the State considering the informations mentioned above. Firstly, four kinds of Key Pests were made clear out; two species of mealy-bug, a species of pyralid, a species of longicorn and a species of mulberry scale. Thereafter we have done some obserbation and experiments to make clear out the ecology and to develop control method of these pest insects. Some of the results of observations and experiments mentioned above, we have extracted some countermeasures for control of the Key pests. However, some insoluble problems were remained. Indonesian Experts who were charging pest control were educated about experimental method on ecology and control of the pest insects.

1. Investigation of the pest insects in main moriculture regions.

We have investigated the pest insects in four regions, Soppeng, Enrekang, Wajo and Sidrap. Condition of mulberry field, management and moriculture technique and harvesting methods were also investigated to make clear out relation between field management and outbreak of pest insects. From the results of investigation, following items were extracted.

1) Out line

We have no statistics data of seasonal prevalence of the pest insects. Therefore, we were not able to realize relation between seasonal prevalence and environmental conditions. However, it was considerable that no influence of temperature was on seasonal prevalence of the pest insects in the State. Because monthly average temperature were between 25°C - 28°C in the state (data from Ministry of Agriculture). These temperatures were optimum ones for growth of almost of all insects. In other hand, monthly rainy days and amount of rain fall were quite variable in season and in location. Outbreak of the pest insects was considered to depend either rain or dry season; mulberry

white scale outbreak end of dry season while pyralid outbreak end of rain season to end of dry season.

2) Phase of pest insects

Following insects were observed as Key pests of mulberry tree.

Pseudaulacaspis pentagona (TARGIONI)
Mulberry scale

Maconellicoccus hirustus (GREEN)
Hibiscus mealybug

Pseudcoccidae sp.

Epepeotes plarator (NEWMAN) (Hereinafter referred to as Longicorn E.P.)

Glyphodes pulverulentalis HAMPSON (Hereinafter referred to as Pyralid G.P.)

Some injurious insects were also observed as follows.

A species of Locustidae (Ailopus tumulus FABRICIUS or similar species), a species of Pentatomidae, two species of Chrisomelidae, a species of Scarabacidae, a species of Elateridae and African giantsnail.

No economical damage was observed by these pests. However, gathered nymphs of species of Locustidae were observed to give a serious damage; most of mulberry leaves were eaten by them at Pakatto and Bili-Bili fields in Centre. This result of observation might suggest that alteration of habitat of the insect forced the outbreak of the insect, and the insect was able to change as injurious one.

3) Relation between condition of the field, field management and outbreak of pest insects.

It was general that farmers planted coconuts arround of and in mulberry field. Humidity in the field were easily increased in these field mentioned above, and the humidity condition became a factor of outbreak of mulberry scale. One of suitable example was observed in Sudu in Enrekang. Numerous

coconuts were planted in a field, and harvesting and triming of mulberry tree was irregular, interval between stumps was also irregular and narrow, dead stumps by damage of insects were remained and inter ridges were used as nursery of coconuts. In the case normal field management might be impossible. Then, infestation of mulberry scale, brown lepra and longicorn was observed such a field. In contrast, we have observed well managed field in Datae in Sidrap. Although coconuts were plan planted in the field, air circulation was kept as good condition; trees were controled as semi - low cut training, inter ridges were covered with waste mulberry shoots which might control weeds. Emulsion of Diazinon was used for control of scale insect in the field, and only few trees were infested by mulberry scale.

Thus, it might be sure that shadowy and high humid condition by coconuts or other high trees caused heavy outbreak of mulberry scale. To prevent the outbreak, some countermeasures to maintain air circulation in the field, as well as low cut training and serious triming of trees might be required.

4) Outbreak of key pest insects and back ground of outbreak.

a. Pseudaulacaspis pentagona (TARGIONI)

(Mulberry scale)

This species outbreak from end of dry season te beginning of rainy season. Following fields were damaged seriously showing various factor of outbreak.

(1) Sudu, Enrekang. 20 a, farmer.

Bad training and triming, remained dead stumps after damaged by pest insects, bad weed control, shadowy condition because of coconuts or another high trees. Mulberry scales were observed on most of all stumps, especially heavily observed on stumps under shadowy condition.

(2) Sabbang, Wajo. 50 a, farmer

Mango or other large tree were north side, coconuts were west side. Outbreak was considered to disperse

from near large trees to east and south side. Damage was already expanded widely only three years after plantation. Gontinuous expansion was considerable.

(3) Turlapae, Soppeng, farmer

Similar condition as in (2) was observed. Outbreak was considerable to expand from stumps under shadowy condition near coconuts or another high trees. Serious outbreak was beginning only two years after plantation.

(4) Donri-Donri, Soppeng. Project (Allupangeng)

Large trees were in near centre of the field (2 ha). Outbreak was considered to expand from near the trees to around of field (50 a). Centre region of outbreak (30 a) was heavily infested; parasitism was observed on near terminal of mulberry shoot Plate 1. Outbreak was considered to expand rapidly with in 3 months. The field was not so shadowy and humid. However, mulberry trees were remained without training and triming during two years after plantation. Thus, the pest insects might be able to multiply under favourable condition without receiving artificial confusion. Low density of natural enemy might be also a factor of the outbreak.

(5) Datae, Sidrap, Project.

There was no problem on field management. Outbreak of the pest insects was slightly. However, trees near water running from reeling factory was heavily infested. This observation was considered indicating that the place near water running was high humid which induced outbreak of the pest insects.

b. Maconellicoccus hirustus (GREEN)

(Hibiscus mealybug)

These species also outbroke during dry season and beginning of rainy season. The pest insects distributed widely in the Stage. Real condition of three fields we investigated was as follows.

(1) Pakatto, Gowa, Centre

Percentage of damaged stumps was low (30%). Serious damage was observed integrating on specifial stumps; infested shoot/stump was estimated using Morisita's density index as

$$I\ \delta = 1.754$$

indicating the possibility that damages was not occured at random but integrated on special stumps and special stumps damaged by the pest insects became centre of disperse of the pest.

(2) Tana Bellange, Soppeng, Sub-Centre

Outbreak was widely observed in the field. Namely about 40 a of field situated in north west area (2 ha) was heavily infested; most of shoot were damaged and terminal of shoot was dwarfed forming nod (Plate 2). Although another area of field were damaged widely, this special area were able to distinguish clearly from another damaged area because the damage in this area was so heavy. The field was evenly flat, some of coconuts were in the field, but these trees were considered no influence to alter the condition. It was considered to be very important to make clear out the reason why the special area was infested so heavily, because we could obtain important information for countermeasurement of control. From following investigation and experiment, it was considered that these different infestations might be cause by field management.

(3) Sidrap, Project

The field was well, no shadowy place was observed. Outbreak of mulberry white scale was observed slightly. However, outbreak of mealybug was partialy different. Some area where stumps were done deep triming were observed only few outbreak, while another area where stumps were done slight triming was observed heavy

outbreak. Thus, triming method after harvesting was considered to be effective on outbreak of the pest insects.

c. Epepectes plarator (NEWMAN)

(Longicorn E.P.)

Four species of longicorn, including six spotted white longicorn (Olenecamptus bilobus) were observed in mulberry field. The most injurbious species was Longicorn E.P. The longicorn was also widely distributed. We have investigated at the field of Centre (Bili-Bili), Sidrap (Project), Enrekang (Project), Donri-Donri in Soppeng (farmer). The species of cultivated mulberry was only Morus nigra. The trees were infested mainly main trunk or gross primary branch to die. However, this mulberry species had so high ability of shooting that only few stumps died by the infestation; new shoots pushed out from trunk near root by compensation of the tree Plate 3. In project field in Enrekang, about 70% of the stumps were infested. However, only less than 1.5 % of trees died because of longicorn infestation alone.

The infestation of Longicorn E.P. was observed mostly on trees which were previously infested by mulberry scale and, or lepra. Stalk borrers as like as longicorn were said to infest plants which lost vitality of tree by various factor. From these aspects mentioned above, it was considered that mulberry trees in the region were infested by longicorn after infection of mulberry white scale and, or lepra.

It was suggested that Longicorn E.P. could easily infest the trees which were previously infested by another pest insects and, or diseases. Thus, it might be possible to evade from damage by Longicorn E.P. with previous control of another pest insects and diseases. Death of stumps, by infestation of longicorn was seemed to be slight, but influence to amount of leave harvest which remained insoluble, had to be made clear out.

d. Glyphodes pulverulentalis (HAMPSON)

(Pyralid G.P.)

This species outbreak during end of rainy season and end of dry season (Plate 4). So we could not investigate enough on the pest insect.

5) Summary

a. Mulberry scale

Shadowy and humid condition and condition of low density natural enemy may promote outbreak of the pest insect. Miss management of newly planted field such as remained without training may induce rapid dispense of the pest insect.

b. Hibiscus mealybug

Technique of harvesting and triming concerns on outbreak of the pest insects. Namely damaged buds and shoots, damanged leaves and dwarf branches must be deeply cut off. Deep triming may be able to control outbreak of the pest insects.

c. Longicorn E.P.

Morus nigra, cultivated widely in the region is seemed to die rare by infestation of longicorn alone, because compensation ability of the tree is very high. The trees are considerable to die by secondary infestation of longicorn S following infestation of mulberry scale and, or lepra.

d. Pyralid G.P.

Outbreak of the pest insects was slightly during our investigation. Thus we could not make clear out occurance factor of the pest insect. However, it was considered that meteorological condition might be concerned on the outbreak.

2. Investigations and Experiments for establishment of control method of key pest insects.

The investigations and experiments had intention to establish practical control methods. Therefore, materials were only limitted

as obtainable in the State, or needful in future.

1) Results of investigations and experiments

a. Mulberry scale

Emulsion of Diazinon was used for control of the pest insects. The agent was effective only for nymph but for imago. The most effective agent for fesmale adults is emulsive Petroleum oil (machine oil) alone at present. However, we could not do experiment for ccntrol of the pest insect using the agent because we could not obtain the agent. So, we have investigated natural enemies of the pest insects as a biological factor which might interfare outbreak of the pest insects at the field of Allupangeng in Soppeng. Following natural enemies of the pest insects were observed.

(1) Predators

Symunus hilaris MOTSULSKY
Lady bird beetle (small black)

Thea etentu FABRIGIUS

Yellow lady bird beetle (or smilar species)

(2) Parasites

Eulophidae spp. (two species)
(species names were unknown)

Relations between population density of mulberry scale and that of lady bird beetles on a branch were summerized in Table 1. On main trunk and primary branch, predators were increased depending on density of hosts, while secondary branch did not show smilar relationship. Although population density of the mulberry scale was so high, that of predators was the lowest. So, it was considered that lady bird beetle were not so effective as inhibiting factor on multiplication of the pest insects. Relation between population density of the pest insects and that of parasitic bees was also investigated on branch (mainly primary branch) which were infested by the pest insects with different population density (about 15 cm^2 in area on a branch was observed)

as was shown in Fig. 1. Population density of the pest insects was seen 0.3 - 11.8 individuals/cm^2, showing only less than 10 % of parasitism by parastitic bees, without finding any relation between population density in each insect.

From these results, it was concluded that both population density in predators and parasitic bees were not depend or that of host pest insects, showing no effectiveness on inhibition of multiplication of the pest insect. In the region where serious outbreak was expected, downward pruning method and application of emulsive Petroleum oil (machine oil) must be introducted.

b. Hibiscus mealybug

These species were most injurious pest insects as same as mulberry scale. The pest insects outbroke September - November 1981 in field of Tana Bellange, we could do investigations on the ecology and experiments for control of the pest insects using the field.

(1) Investigation on behavior of injury

(a) Investigation on real condition of injury: To make clear out the injured portion on a shoot, vertical distribution of injured the leaves were investigated. Results of observation on damaged shoots (50 - 130 cm in length) showed that damage of leaves was 61.3% in upper portion, 33 % in middle portion and 5.7 % in lower portion. The results indicated that leaves in upper portion of shoot were easily infested and injured by the pest insects. Namely, on the shoots which leaves were injured more than 50 % showed heavy dwarf of terminal bud, mostly as like as nod, stopping the growth, serial damage of leaves (10 - 15 serial leaves) (Fig. 2). Similar phenomena were observed in field where outbreak was no so serious such as in Pakatto.

(b) Moving ability of female adults: The purpose of the

experiment was to estimate migration and disporsal ability of the pest insects in field. The female adults were collected from mulberry field and put on the paper situated out door under sunny condition or on the paper situated in door under shadowy condition, and duration and distance of migration of the pest insects on paper were observed. Duration and distance of migration were only about 18 min and about 140 cm under sunny condition, while they migrated about 95 min, 830 cm under shadowy condition, suggesting that the pest insects could migrate and disperse so widely.

(c) Host selection by female adults: Host preference by adult females was examined using terminal leaves, grown leaves and branches of newly shoot, showing no significance on host selection.

(c) Behaviour on mulberry shoots and field: On the shoots larvae parasited mainly on terminal bud, while female adults parasited everywhere. Namely, matured female adults situated themselves where they could hide; inter space between petiole, stiple and stem, behind of dwarfed leaves, etc. From in door observation, we considered that the mealybugs could disperse more than one metre, searching easily terminal bud where was optimum habitate. However, we could not observe clearly migration from terminal buds which were situated in centre of inter ridge space to mulberry tree (about 70 cm of distance from centre of inter ridge to ridge of trees). Therefore it was considered that some factor inhibiting migration of the pest insects might be exist in the field.

(2) Experiments on control method

Combination of cutting back after harvesting and application of insecticide was examined to obtain basic data for control of the pest insects.

Materials and methods: Experiments were done from 25th

September 1981 when was beginning of outbreak. Experiments were refrained three times as following.

Experiments	Insecticide Applicated	Dilution	Amount of Application L/ha
A. Cutting back	-	-	-
B. Cuttingback + Insecticide	Phenthoate (PAP) Emulsion	x1,000	1,000
C. General triming + Insecticide	"	"	"
D. General triming (Check)	-	-	-

Cutting back was done as all leaves and buds which mealybugs could parasite were eliminated, while general triming was done as some leaves, buds and dwarfl shoots remained.

Heavy rain fall (about 30 mm, one and half hours) stacked about 3 hours after spraying the insecticide, we had to spray the insecticide again next day for addition, 500 L/ha. Results were observed 18 days and 34 days after treatment. Dwarfed and noddule formed buds observed on treated trees were less than that of on no treated control, showing effect of the treatments on control of damages by the pest insects. Combination of cutting back and insecticide was slightly better than another treatment, but no significant difference (Fig. 3, Table 2). Namely, each treatment showed that damages buds decreased more than 90% compared no treated one. From results mentioned above it was suggested that cutting back for including latent buds was very effective to prevent damages by the pest insects.

c. Longicorn E.P.

Although outbreak of the species was wide spreaded and percentage of injured stumps was high in a certain regions, it was considered that damages by the pest

insects were not so serious as was describe above. However, it was also considerable that outbreak of the pest insects might increase after alteration of moriculture technique and mulberry variety, application of high amount fertilizer, introduction of Morus alba or another variety, etc. The experiments were done to establish control method applicable in future. Materials and methods: Mulberry field of Centre in Bili-Bili and in Enrekang (Project) were used for the experiment. The insecticides used were Methidathion (Spuraside emulsion) and Fenthion (Toraside emulsion) which were using in Japan for control of longicorns. The chemicals were diluted as 50 times and 100 times, and were sprayed 1,200 L/ha.

Result: As was shown in Table 3, density of larval population was not so high that we could not estimate effect of the insecticides to the pest insects. Both insecticides were considered that spray of 50 times diluted emulsions as 1,200 L/ha was not enough for control of the pest insects. Experiment at field in Enrekang showed no effective data because of the lowest density of larvel population.

d. Pyralid G.P.

Outbreak of Pylarid G.P. was very few during our experiment. So we had to do preventive experiment on outbreak of the pest insect, using farmers mulberry field in Donri-Donri in Soppeng. However, we could not obtain preventive effect of insecticides tested (Fig. 4). Thus, control of the pyralid was considered to be done during larval stage.

2) Summary

a. Mulberry scale

In the field where serious outbreak was observed, we could not find out natural regulation of the pest insects; predator and parasitic enemies were not so much. In the case, it was considered that we had to introduct some effective

methods for control the pest insects, emulsive Petroleum oil (machine oil) low cut training for example.

b. Hibiscus mealybug

Cutting back for inducing latent buds might be avairable method to prevent the outbreak. Combination of cutting back and application of insecticide might give the most effective results for control the pest insects.

c. Longicorn E.P.

Our experiments on application of insecticide for control the pest insects could not make clear out the effects.

d. Pyralid G.P.

Preventive method for control the pest insects was not available. Countermeasure might be better taken under larval stage of the pest insects.

III. PROBLEMS AND COUNTERMEASURES

1. On the control of pest insects in mulberry field at present.

1) General discussion

Regarding meteorological and environmental condition and field management, it was considered that mulberry fields in the State had some factors which might induce easily serious outbreak of pest insects. In addition, lucking of materials for control, miss management on field maintenance and utilization planning, and lucking recognization of farmers for control the pest insects, countermeasures for control of the pest insects was considered to be difficult doing immediately. However, suitable field management may be able to replace afore going countermeasures, planting distance (interspace between each ridges and each stumps), planting escaped from shadowy places, weed control, suitable training and triming, field maintenance including rearing plan and field utilization plan, etc, for example. To accomplish these management, minimum tools may be required; a pruner (scissors special for pruning and triming of mulberry trees) and a saw for pruning. These tools may be avairable to eliminate dead branches and shoots after parasitism of pest insects or parasitic microorganisms, and dwarf shoots where pest insects and pathogens can hide and disperse to another healthy parts. Nextly, sprayer of insecticides may be also required. If each farmer can not obtain their own sprayer, cooperative utilization may be better for introduction of sprayers. For insecticides, it was considered that insecticides obtainable in the State might not enough for control of mulberry pest insects. For example, emulsive Petroleum oil, the best countermeasure for scale insects may be better to introduct in the State, by import or fabrication in the State. However, fundamental countermeasure for control of the pest insects is a suitable field management including utilization planning as mentioned above, and utilization of insecticides is only a part of supporting method for field management.

Application of insecticides can be allowed only few times using as possible as low concentration.

2) Practical countermeasures for control of key pest insects.

a. Mulberry scale

The pest insects can multiply rapidly under shadowy and humid condition. Inter space between each ridge and each stupm must be widered enough (more than 1 m between ridges, and 50 cm between stumps). Weed control must be done well. These countermeasures are to obtain well air circulation in the field. Plantation of mulberry trees in shadowy place, under coconut trees for example, must be avoided. Because the pest insects can easily outbreak under these condition and disperse to another healthy place. In field where serious outbreak is expected, low cut pruning must be done, and triming and pruning must be done well for decrease the population density. The best insecticide for control the pest insects is emulsive Petroleum oil. Introduction of the chemical may be required in future. When using Diazinon, the chemical must be sprayed 2 - 3 times at 7 - 10 days interval after triming, because the chemical is effective only on larval stage.

b. Hibiscus mealybug

As already described, outbreak of the pest insects is related to skilfulness of triming and pruning. Cutting back and triming must be done well in fields where outbreak is observed always or expected. Spray of some insecticides after cutting back or triming may be avairable for decrease of population density in serious damaged field. Female adults are considered to be able to migrate and disperse several metre. Therefore, shoots and leaves after cutting must be eliminated from arround of stumps. These waste shoots and leaves must be burnt out or accumulated at where is enough distanced from the field.

c. Longicorn E.P.

Economical damages by longicorn was considered to be slight, because Morus nigra, common species in the State had

strong compensation ability for damages by Longicorn E.P. Low cut pruning may be required at the field where serious outbreak is observed. In addition, introduction of new variety of mulberry must be done carefully considering outbreak of the pest insects.

d. Pyralid G.P.

Control of the pest insects with agronomic method may be difficult. We have only to recomend application of insecticides for the pest insects. However, farmers must plan harvest schedule because application of insecticides must be done occasionally in rearing times. If farmers want to harvest in early times after application, the chemicals which have low residual toxity, such as emulsive Dichlorvos (DDVP, within two days), (Naled Dibrom, within seven days), must be used. In another hand, Trichlorfon (Dipterex, within twelve days) and Phentoute (Papthion, within seventeen days), may be avairable for control the pest insects.

2. Countermeasures as research which must be done immediately

1) General

Investigationon life cycle and seasonal prevalence of key pests.

2) Mulberry scale

(1) Realization on mechanism of disperse of damages (outbreak)

(2) Investigation on possibility of Biological Control

(3) Screening of effective insecticides and investigation on practical method of chemical control.

3) Hibiscus mealybug

(1) Investigation on injury habitat.

(2) Investigation on relation between damages and parasitic population density and parasitic period.

(3) Estabilishment of control technique combined with agronomic method.

4) Longicorn E.P.

(1) Investigation on relation between nature of secondary stem and egg raising.

(2) Screening of avairable insecticides and method of application.

5) Pyralid G.P.

(1) Investigation on relation between meteorological condition and amount of outbreak and occurance period.

(2) Application method of Dichlorvos, Naled, Trichlorfon, Phenthoate and Diazinon (Concentration, Application period, amount of application).

IV. SUMMARY (GENERAL)

It might be surely said that mulberry fields in South Sulawesi State was situated under shadowy and humid condition, without doing complete triming. These condition might induce serious damages by pest insects. Four kind of key pest insects were recognizable at present, some of them were not yet identified genera, species and their life cycle. Following countermeasures were considerable for control these pest insects.

1. Pseudaulacaspis pentagona (TARGIONI)

Shadowy and humid condition induce the outbreak. Therefore, a suitable field management is necessary for control the pest insects. For chemical control, emulsive machine oil is the best. Import or Fabrication of the chemicals in Indonesia may be desired as countermeasure.

2. Maconellicoccus hirustus (GREEN)
Pseudococcidae Sp. (Hibiscus mealybug)

Cutting back after harvesting is the most practical method for control the pest insects. Screening of applicable insecticide may be also required. In addition, the life cycle, behavior of host preference, injure and appearance of damages must be made clear out for establishment of control method.

3. Epepeotes plarator (NEWMAN)

It seems that there are no problems or damages on Morus nigra by the pest insects, because this mulberry species has high compensation ability, and the trees are rare to die with damages by the pest insects. However, we could not relize damages by the pest insects on another mulberry species. We must investigate carefully outbreak of the pest insects after introduction of new mulberry species such as Morus alba.

Screening of insecticides applicable to the pest insects may also be required.

4. Glyphodes pulverulentalis (HAMPSON)

Application of insecticides is the most avairable method for control the pest insects. For application of insecticides, planning of field utilization, technique for prediction of occurance and realization of effective insecticides may be required in early time.

Table 1. Relation between number of the predator and population density of the mulberry scale at Alupangeng. (28, Sep. 1981)

Part	Grade of population density of mulberry scale	No. of trunks or branches examined	No. of predator (larvae)	No. of predator per one branch
Trunk	H	2	1	0.50
	M	7	2	0.29
	L	12	0	0
Primely branch	H	23	7 (1)	0.30
	M	49	13 (1)	0.27
	L	50	2	0.04
Secondly branch	H	49	7 (2)	0.14
	M	77	43 (33)	0.55
	L	52	7 (2)	0.13

Remarks: H : More than 5 individuals per one cm^2
M : 2 - 5 individuals per one cm^2
L : Less than 2 individuals per one cm^2.

Table 2. Effectiveness of different treatment for control of the hibiscus mearlybug at Tana Bellange.

Treatment	Number of shoots per plant	No. of damaged shoot (dwarf bud)		Damaged shoot ratio of check	
		Oct. 14	Oct. 30	Oct. 14	Oct. 30
Cutting back	59.5	3.9 (0.4)	4.4 (1)	-72.9(-93.7)	-75.4(-91.3)
Cutting back + Insecticide application	70.4	3.8 (0.2)	3.8 (0.7	-77.7(-96.7)	-82.0(-95.5)
General triming + Insecticide application	63.5	5.5 (0.7)	4.7 (1.2)	-58.7(-90.3)	-75.5(-91.3)
General triming (Check)	53.4	12.9 (5.4)	16.0 (11.2)		

Table 3. Effectiveness of different insecticides for control of hibiscus mulberryborer at Bili-Bili

Insecticides	Dilution	No. of plants examined	No. of larvae					
			Healthy			Dead		
			Y	M	O	Y	M	O
Methidathion (Supracide)	50	10			1[A]	1[*]	1[*]	
	100	7	1[*]	1	2			
Fenthion (Torasaide)	50	5	1[*]	4[*],2		3[*]		
	100	5	2[*]	1	1[PP]	2[*]	1[*]	
(Check)		5		2[*]	1			

Remarks: Y : Young stage M : Middle stage O : Old stage

A : Adult PP : Pre Pupa

* : Larvae leaved in bark.

Table 4. Effectiveness of different insecticides for control of the mulberry pyralid at Soppeng

Insecticides	Number of plants examined	No. of shoots per plant		
		Total no. of shoots	Damaged shoots	Larvae
Dichlorvos (DDVP) x 1,000	22	41.0	7.6	4.7
Salithion x 1,000	22	37.2	5.3	3.6
Phentoate (Elsan) x 1,000	22	37.0	4.9	3.8
Check I	25	30.2	6.4	4.4
Check II	10	36.9	3.5	3.4

Plate 1. Severe injury to mulberry plant caust by mulberry scale, Pseudaulacaspis pentagona (TARGIONI).

Plate 2. Mulberry bud damaged caused by hibiscus mealybug, Maconellicoccus hirustus (GREEN).

Plate 3. Mulberry branch damaged caused by longicorn, Epepeotes plarator (NEWMAN).

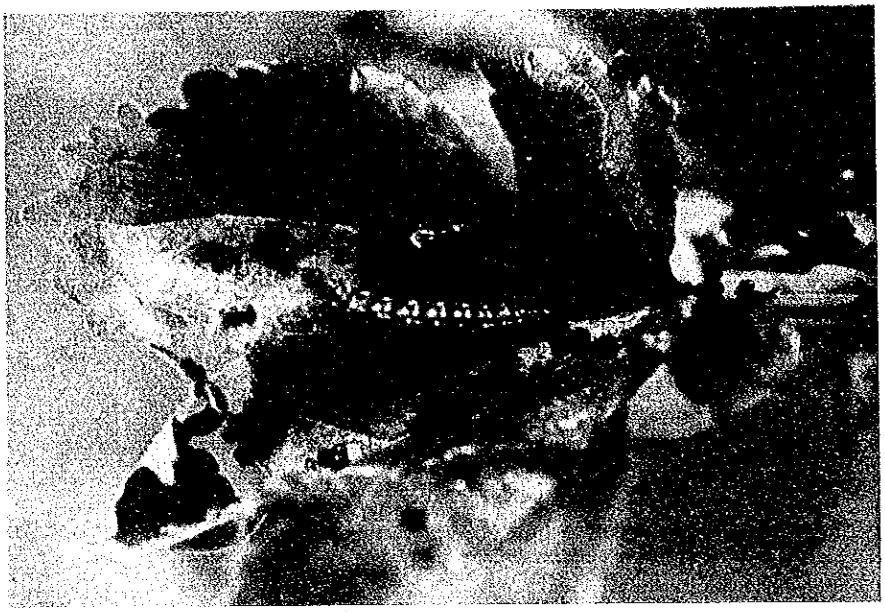


Plate 4. Larva of Glyphodes pulverlentalis HAMPSON and damaged leaf.

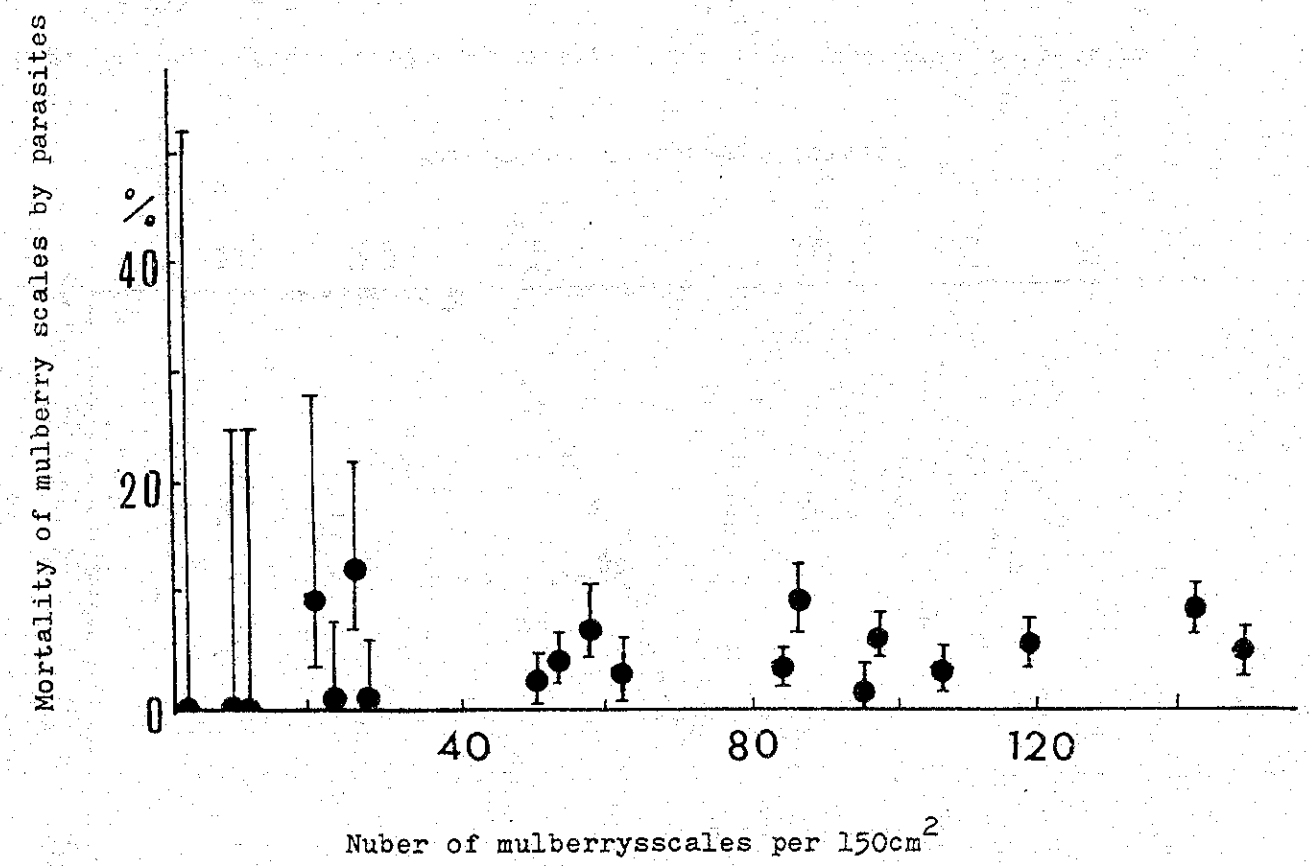


Fig. 1. Relation between the number of white scale and percentage parasitism.

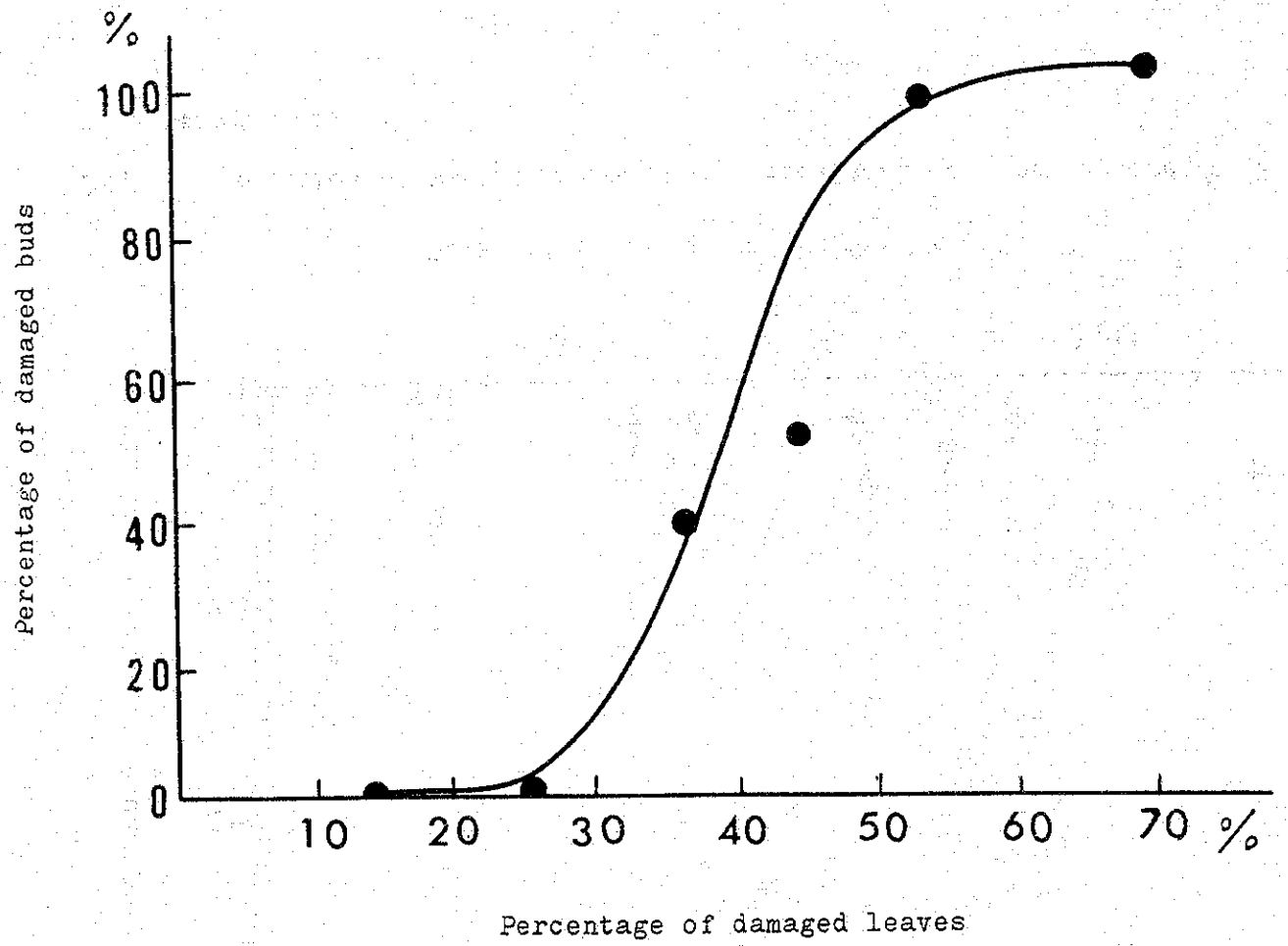


Fig. 2 . Relation between percentage of damaged buds and percentage of damaged leaves.

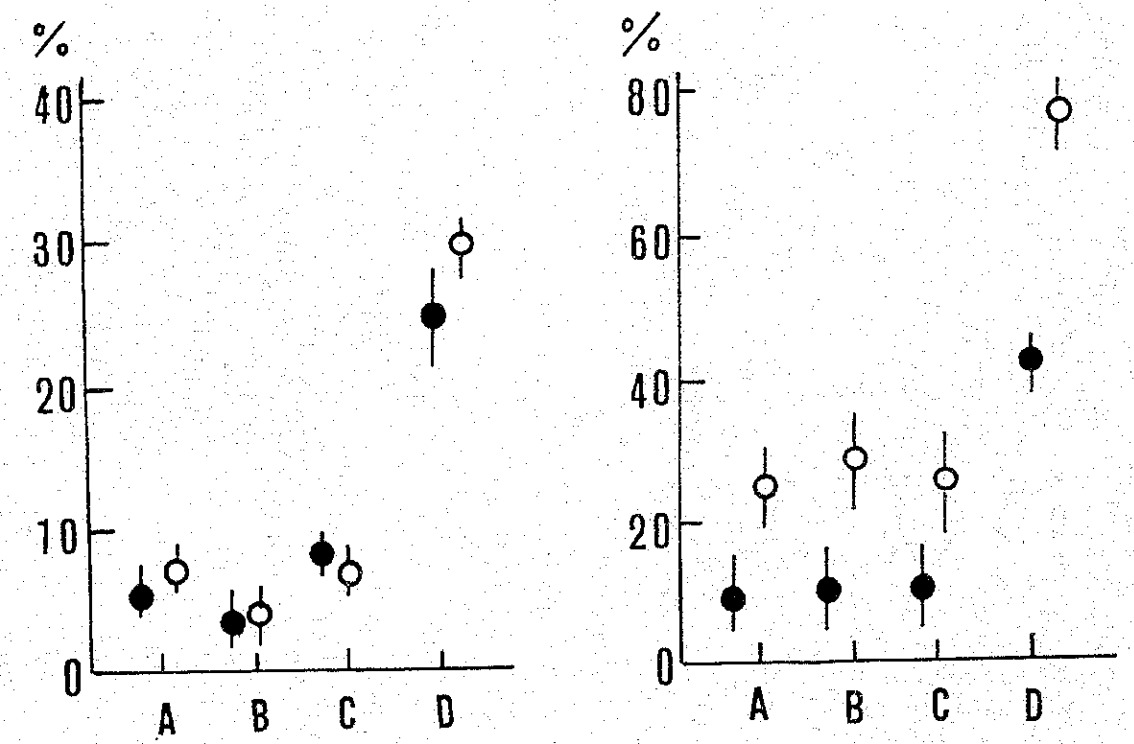


Fig. 3. Effectiveness of differenttreatment for control of the mealybug.

Left : Percentage of damaged shoots, Right : Percentage of damaged buds.

A : Cutting back

B : Cutting back + Insecticide application

C : General triming + Insecticide

D : General triming (Check)

—●— : Oct. 14 , —○— : Oct. 30 investigated.

C. 土壌専門家報告書（和文）

南スラウェシ州における桑園土壌調査

早　坂　　猛

派遣期間

昭和57年3月26日～

昭和57年6月25日

はじめに

1982年3月28日から約3か月の日程で南スラウェシ州に滞在し，養蚕プロジェクトビリビリセンターにおいて，すでに供与されていた研究用機材を使用しながら，土壌肥料分野のカウンターパートの養成を兼ねて，各地の桑園土壌断面調査ならびに土壌分析を行なった。本報告ではこれらを研修と土壌調査の2篇に分けて記述する。

第1の研究スタッフに対する研修は，土壌調査法，土壌の物理分析，土壌の化学分析の3つに分け，全体を通してはカウンターパートのスチブト，また，重要な部分についてはエンジャン，ムナッサールの両カウンターパートを加えて説明と実習とを行なった。この部分は土壌調査に関するインドネシア語による研修テキスト作製の問題を念頭において記述するので，報告という性格にそぐわない面があることを，あらかじめお許しいただきたい。しかし，取扱った内容は今回実施することのできた調査・分析項目に限っており，報告から大きく逸脱することを避けた。したがって止むを得ず実施できなかった項目については，テキスト作製の際に追加されるべきである。

第2の南スラウェシ州主要養蚕地帯における桑園土壌調査成績は，ビリビリの養蚕センターとパカトー，マリノーの附属桑園を除いては，ソッペン，ワジョー，シドラップ，エンレカン県の養蚕地帯について行ったもので，概括的なものではあるが地域の特性を捉えることを目的としたものである。これらについて，一部分析が終了していないが，土壌断面調査ならびに土壌分析成績をもとに，土壌診断ならびに土壌管理対策について述べる。

I　桑園土壌調査法ならびに土壌分析法

別に詳しい内容の報告を残したが，繁雑になるので，記述した項目だけをここに示すことにした。

1. 桑園土壌調査法

1－1　桑園土壌調査

1－1－1　試坑の作り方

1－1－2　土壌断面調査

1－1－3　試料の採取

1－2 桑園土壌の分類

2. 土壌の物理分析法

2－1　採土法

2－2　実容積測定法

2－2－1　原理

2－2－2　測定法

2－3 仮比重

2－4 真比重

2－5 固相率

2－6 液相率・気相率

2－6－1　PFの概念

2－6－2　測定法

2－6－3　PF 1.5 飽水度

2－7 有効水分

2－8 透水係数

2－8－1　ダルシーの法則

2－8－2　測定法

3. 土壌の化学分析法

3－1 分析試料の調製

3－2 pHの測定

3－2－1　pHメーターの使用法

3－2－2　土壌pHの測定

3－3 置換酸度

3－3－1 理論

3－3－2 酸・アルカリ・塩の濃度

3－3－3 中和滴定法

3－3－4 置換酸度 Y_1 の滴定

3－4 置換性塩基

3－4－1 試 薬

3－4－2 方 法

3－5 全窒素（T－N）

3－5－1 湿式分離法

3－5－2 セミミクロ蒸溜装置の使用法

3－5－3 土壌の全窒素定量法

3－6 無機態窒素の分析法

3－6－1 微量拡散法

3－6－2 乾土効果

3－7 有効態リン酸・リン酸吸収係数

3－7－1 比色計の使用法

3－7－2 アミドール法によるリン酸定量

3－7－3 バナド・モリブデン酸法

3－7－4 有効態リン酸

3－7－5 リン酸吸収係数

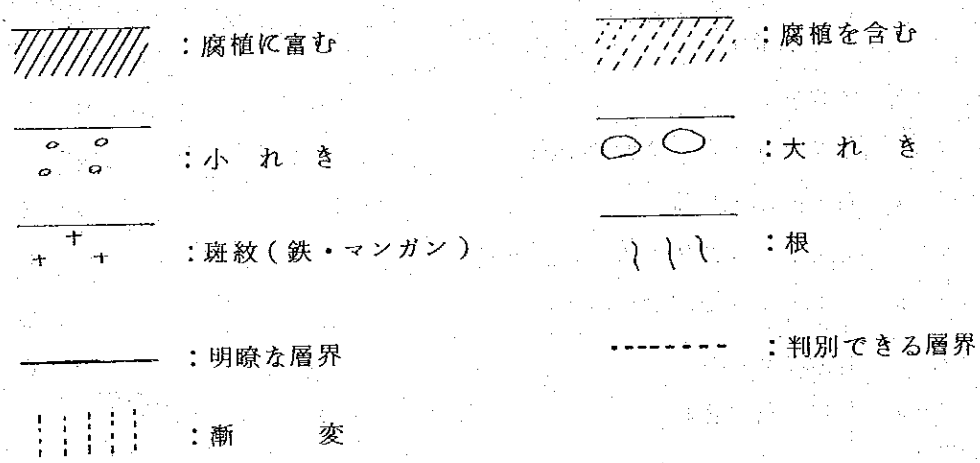
Ⅱ　桑園土壌調査

1　各地桑園土壌調査の概要

桑園の土壌調査を行なったのはビリビリの養蚕センター桑園，同バカトー桑園，同マリノー桑園，ソッペンサブセンターのタナブランゲ桑園ルパンゲ桑園のほか，ナショナルプロジェクト関係でマサッペ，バラッカ，スズ，さらにパイロットユニットのウギとワニヨの合計 10 か所である。また，タナブランゲでは新植予定地，バカトーでは肥料試験区の表土の窒素成分の比較調査を行なった。

これらの各桑園の調査成績を個別に述べるが，バラッカ，スズ，マリノーの 3 か所は分析がまだ終了していない。

次に全体を通じて共通に使用した記号について述べる。土壌断面のスケッチでは次の記号を使用した。



物理分析においては，ルパンゲ，ウギ，ワニヨ，マセッペならびにビリビリについては，乾燥時にスイッチ操作の誤りで異常高温となったため，ピクノメーター法による真比重を使ってデータの修正を行なった。

分析項目の中で仮比重は 0.9 ～ 1.2 が良好，1.3 ～ 1.5 が不良 1.6 以上は耕作不適と考えられる。これは固相率の 33 ～ 45 %，50 % 以上，60 % 以上に当る。また気相率は粗孔隙に当るもので 15 % 以下はやや不良，10 % 以下は不良である。

液相に関しては全孔隙に対する比である飽水度で示した。これは水分環境が乾燥しているが湿潤であるかの度合を示すと考えられ，50 % 以下は乾燥型，60 ～ 70 % が適潤型，80 % 以上を過湿型としてわけてみた。また，飽水度を質の面とすると，有効水力は供給量を示すもので，8 % 以下が不足，8 ～ 10 % が普通，12 ～ 16 % が良好，16 % 以上優良と考えた。

透水係数は 10^{-2} のオーダーが過良，10^{-3} が良好，10^{-4} やや良，10^{-5} やや不良，10^{-6}

難透水，10^{-7} 以下を不透水と考えた。

化学分析成績の項目の中で置換酸度については，3～6がやや酸性，6以上を酸性とした。

カルシウム，マグネシウムは，それぞれ7m.e（ミリ当量），1m.e 以上あれば良いとした。全窒素は耕土層で100㎎％以上含まれることが望ましい。また有効態リン酸は10㎎％以上が良好。2㎎％以下を不良と考えた。

灼熱損失量は，風乾土をガスバーナー上で20分間灼熱し，その重量の減少量を170℃乾燥の乾土に対して比較した。170℃は有機物が分解しない最高温と考えられる。しかし，鉄やマンガンを多く含む土壌では，これらの酸化による重量増が起るが，タナブラングやルパンゲでは，最下層で著しく現れた。そういう解釈の難かしさがあるため，参考値として添えた。

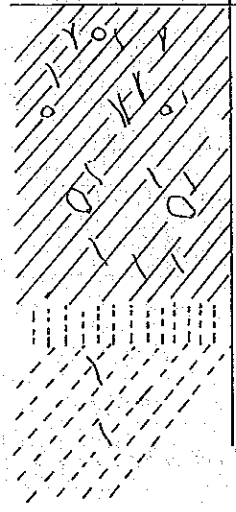
1）ビリビリ桑園

養蚕センター構内の桑園で丘の南斜面の中腹の自然テラス上に設けられた桑園である。母材となっているのは，第三紀の火山活動に伴なう熔岩であるようだ。しかも他と比べて孔隙に富んでいるようであり，浮石あるいは凝灰岩質のものも入っている可能性がある。畑面に石が散在している割には土層中のれき含量は少なかった。土壌断面調査の概要はTable 1-1 に示すとおりである。

この表の中で特徴的なことは，排水が良好で斑紋がみられないところへ表層にだけ小さなれきの風化物が鉄の斑紋ように赤い斑点として散在していることで，これは火山起源の浮石あるいは岩滓かと思われた，そして土塊が全層にわたってほぐれやすい粒状構造を持ち，物理的にすぐれているようであった。腐植もかなり含まれている様で，根の分布も多く，優良桑園であると考えられる。

Table 1－1　土壌断面の特徴

ビリビリ

土壌断面	層位 深さ cm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	極暗赤褐色	埴壌土	風化れき斑紋	軟粒状	24 ㎜	細～中とむ 10 %
	35 cm						
	2	暗赤褐色	埴壌土	な　し	軟粒状	22	細小含む 5 %
	75						
	3	暗赤褐色	埴壌土	な　し	軟粒状	22	細あり 2 %

土壌の物理分析の結果はTable 1－2のとおりであるが，第1～第3層まで全く同様の性質を示し，仮比重が1よりやや高い程度で，固相・液相・気相のバランスも良く，土壌の湿潤条件（飽水度）も旱害の可能性が低く，有効水分の保持力も比較的高くで，物理的には極めて良好である。

Table 1－2　土壌の物理分析成績

ビリビリ

層位	仮比重	pF 1.5 三相分布%			全孔隙%	飽水度 pF 1.5%	有効水分%	透水係数
		固相	液相	気相				
1	0.998	38.4	43.0	18.6	61.6	69.8	15.2	3.4×10^{-3}
2	1.043	39.3	43.3	17.4	60.7	71.3	12.5	5.0×10^{-3}
3	0.937	34.6	53.4	18.8	65.4	81.7	13.3	4.2×10^{-2}

化学分析の結果はTable 1–3に示したが，pHのところで，pH（H_2O）よりpH（KCl）の値が大きいという異常がみられる。他の地点でも同様のことがあるので，ガラス電極の劣化で不安定になったのかもしれない。第2・第3層はPHが低いものの置換酸度は小さく，N－KCl可溶CaO，MgOも多いので酸性土壌ではないようだ。とくにマグネシウムが多いのは塩基性岩に由来するためであろう。しかし全窒素は非常に少ない。また有効態リン酸もやや不足である。

Table 1－3　土壌の化学分析成績

ビリビリ

層位	pH		置換酸度 Y_1	N–KCl 可溶 m.e		全窒素		リン酸		灼熱損失%
	H_2O	KCl		CaO	MgO	mg	%	有効態mg	吸収係数	
1	6.2	5.0	0.7	17.0	9.7	65.6		2.5		5.65
2	4.6	5.0	0.7	14.8	8.1	70.7		2.4		4.16
3	4.6	4.8	2.2	15.3	10.9	4.2		1.2		4.06

対策としては窒素の含量が低いので肥料によって補うことが，何よりも必要である。他の点についてはすぐれた性質を持つ土壌であるから，施肥の効果は出やすいはずである。有効態のリン酸も不足してはいないが，施肥としてはリン酸も加えてバランスをとるべきであろう。

2）パカトー桑園

ビリビリのセンター構内の桑園に比べて約100m低い沖積平野に開かれた桑園で北側にやや高い丘が連なる。沖積土の特性としてTable2－1に見るように土性のちがう層位が明らかな

互層となっており，円れきが含まれる。中には明らかに軽いれきがあり，ピリピリ同様，浮石あるいは凝灰岩質の母材が入っているようである。また集塊岩あるいは礫岩質の暗色のれきもあり，複雑な様相を示している。断面の特徴としては第2，第3層に鉄・マンガンの斑紋があり，雨期に停滞水を生じるものと考えられるが，この水の浸透をさまたげる第3層の砂質層は，

Table 2－1　土壌断面の特徴

パカトー(1)

土壌断面	層位 深さ cm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	暗赤褐色	埴壌土	なし	細粒状	16 mm (山中式)	細～中富む
	20cm						
	2	暗赤褐色	埴土	Fe, Mn 雲状斑	細粒状	18	細富む
	50						
	3	暗赤褐色	砂壌土	Fe, Mn 雲状斑	塊状	24	細あり
	90						
	4	にぶい赤褐色	埴土	なし	粗粒状	13	
	100+						

乾期には地下水からの水の供給を断ち切る働きをするので，近辺に小川があるにもかかわらず，桑が旱害を受けるおそれがある。第2層が埴土でありながら細根に富むことは，常識とは異る点である。全体として土色が赤く，腐植は少ない。

物理分析の結果では第2層の仮比重が著しく小さく，気相（粗孔隙が多いことが特徴である。見かけ上の第3層よりも，この軽い第2層の方が水を停滞させるのではないかと推定されるが，第1層の湿潤度（飽水度）が高いのは，このためと考えられる。それと有効水分の保持力が小さいことが問題点である。

Table 2－2　土壌の物理分析成績

パカトー(1)

層位	仮比重	pF1.5 三相分布 % 固相	液相	気相	全孔隙%	飽水度 pF1.5%	有効水分%	透水係数
1	1,156	44.6	45.0	10.4	55.4	81.2	7.4	6.9×10^{-4}
2	0.946	35.2	45.2	24.7	64.3	69.8	6.8	2.0×10^{-3}
3	1,123	41.6	42.0	16.5	57.3	71.9	7.1	1.3×10^{-3}
4	1,143	43.6	44.2	12.2	56.4	78.9	10.7	3.5×10^{-4}

Table2–3に示した化学分析成績で第3層のpH（H_2O）がpH（KCl）より低い上，全層の中でもpHが低くなっている。これはpH（H_2O）の測定誤差であろう。置換酸度とCaO，MgOの含量の高さからそのことが推定できる。とくにマグネシウムが多いことは，近くのビリビリ桑園と共通であり，また窒素含量が低いことも同様である。

対策としては，水分供給力の弱さを補う点と，窒素主体の施肥であるが，施肥は問題ないとしてよいであろう。水分供給力では，基本的には互層になっているのを天地返しで均一化すれば改善されると考えられるが，さし当っては稲わらなどによるマルチがよいであろう。

Table 2－3　土壌の化学分析成績

パカトー(1)

層位	pH		置換酸度 Y_1	N—KCl可溶m.e		全窒素	リン酸		灼熱損失
	H_2O	KCℓ		CaO	MgO	mg %	有効態mg	吸収係数	%
1	6.6	5.0	0.7	19.8	12.8	74.4	10.1	2,346	3.65
2	6.2	4.6	2.3	17.1	14.3	42.2	7.7	1,325	2.82
3	4.6	4.7	1.6	20.0	12.5	69.6	5.1	2,417	2.24
4	5.1	4.7	1.4	16.3	15.4	52.6	6.3	2,356	2.19

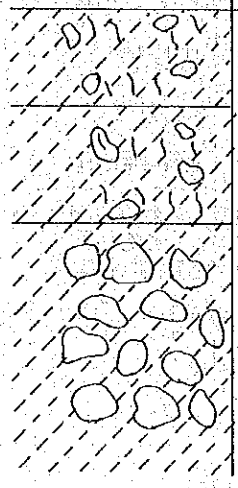
3）マリノー桑園（造成前）

パカトー，ビリビリを経てさらに東へ進んだ山岳地帯にマリノー桑園が造成されつつある。標高約1000m といわれ，西へ向って5°程度のなだらかな傾斜地である。周辺は森林がよく発達しており，肥沃な地帯に近いであろう。樹種に松が多いところから，水分係給はあっても栄養的にはやや劣るかも知れない。

土壌断面の概要はTable 3－1 に示したが，母材は第1・第2層は火山灰であり，第3層は凝灰岩質の大きなれきが多く含まれ，土壌化した部分は表土に比べて重い土である。れきは割れやすく，風化しやすいと思われるが，高い場所では表土にもれきが多く，また低くなっている場所は表土が流れてたまったとみられ腐植に富んでいる。最後に調査した地点で分析が完了していないので詳しくは言えない。

Table 3－1　土壌断面の特徴

マリノ

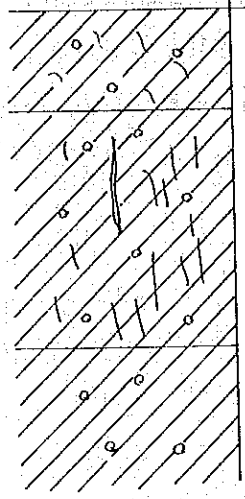
土壌断面	層位深さ cm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	暗褐色	埴壌土	な し	軟粒状	20 mm	草の根富む
	20 cm						
	2	暗褐色	埴壌土	な し	軟粒状	22	同 上
	38						
	3	黒褐色	れき土(埴壌土)	な し	軟粒状	21	な し
	100+						

4）タナブランゲ桑園

南スラウェシ州中央部のテムペ湖を中心とする低地の穀倉地帯の一部で，沖積土であるが，湖沼性の堆積物を母材としているようである。Table4－1に示した土壌断面でも土性は一様に埴土で，黒く着色していて腐植に富む様に見える。しかし，土壌の分析試料を焼いても黒さが残ることから，マンガン酸化物による着色である可能性も強い。表層から斑鉄が現われていることから，雨期には水が停滞すると思われるが，こういう湿害の現れそうな土壌としては意外な程深く桑の根が入っており，乾期のひび割れに沿って根が入ったものとみられるから，長い間には構造の発達が起る可能性もある。内部排水を促進する必要がある。

Table 4 － 1　土壌断面の特徴

タナブランゲ(1)

土壌断面	層位深さ cm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	黒色	埴土	点状斑鉄あり	粗粒	18 mm	細あり
	12 cm						細～中含む
	2	黒褐色	埴土	なし	堅果クラックに富む	19 mm	クラックに沿って
	80 cm						
	3	黒褐色	埴土	なし	角塊	17 mm	細あり
	100+						

土壌の物理分析の結果はTable4 － 2のとおりであるが，とくに第3層の仮比重が 1.3 をこえ，固相率も 50 %以上で，気相率が(－)値を示している。これは土壌中に液相に囲まれた気泡が封入されたためであるが，いずれにしても粗孔隙が極端に少ないことに変りはない。この第3層に対して第2層，第1層と表層に向って物理性は良くなっている。しかし有効水分の保持量が非常に小さく，干害にかかりやすいものと推定される。

Table 4 － 2　土壌の物理分析成績

タナブランゲ(1)

層位	仮比重	pF 1.5　三相分布　%			全孔隙%	飽水度	有効水分%	透水係数
		固相	液相	気相		pF 1.5 %		
1	1,058	38.9	39.8	21.4	61.1	65.7	5.1	3.8×10^{-4}
2	1,225	47.4	43.8	8.7	52.6	83.2	5.1	6.1×10^{-5}
3	1,358	52.5	48.8	(−1.2)	47.6	102.7	5.7	3.7×10^{-3}

化学分析成績をTable4 － 3に示したが，石灰岩質の母材による土壌と思われ第1層がやや低かったもののpH(H_2O)はアルカリ性に傾いていた。置換酸度は僅かであり，N－KCl 可溶のカルシウム，マグネシウムとも著しく多く，有効態のリン酸も多い。これに対して全窒素の含量は第2・第3層において著しく低い値を示した。

Table 4－3　土壌の化学分析成績

タナブラング(1)

層位	pH		置換	N−KCl可溶me		全窒素	リン酸		灼熱損失%
	H_2O	KCl	酸度Y_1	CaO	MgO	mg%	有効態mg	吸収係数	
1	6.0	6.5	0.3	29.3	5.3	73.3	16.4	2,310	2.02
2	8.2	6.8	0.3	35.6	6.2	30.9	14.5	2,454	0.44
3	8.2	6.8	0.8	33.9	10.0	132	191	2,676	—

土壌改良の重点は物理的不良性の改善にあるが，当面は水分不足を補い，また有機物を供給するということでわらマルチを行なうことが望ましい。一年を通して気温が高いため，マルチがが害虫の繁殖を促すことにはならないと考えられる。

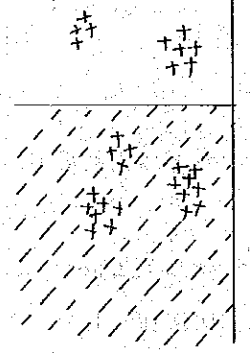
造成予定地（タナブラング2）

同じ桑園の西側の隅に原野が残されており，一部には桑が植えられていたが，生育は極めて悪かった。

この原野の中心近くを40 cm 程掘って，簡単な調査を行なった。生育不良を起している区域の地表に水が浸み出していることから排水不良であると推定されたが，Table4－4に示した断面調査にもそれが現れており，表土から斑鉄が出現していた。また，コンシステンシーの値が表土から20 mm 以上で，かなり耕起するのに固い土であるといえる。

Table 4－4　土壌断面の特徴

タナブラング(2)

土壌断面	層位 深さcm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	灰黄褐色	微砂質壌土	雲状斑鉄	堅果状	24 mm	草の細根富む
	20cm						
	2	黒褐色	埴土	雲状斑鉄	角塊	23	
	35+						

物理分析の成績はTable4－5に示すとおり，タナブラング既設桑園の第2層とよく似ており，固相率約50％で重い土であり，湿潤度を示す飽水度が80％以上で，常に湿潤であることを示している。有効水分が少ないから，桑を植えると湿害と干害の両方に苦しむことになるであろう。

Table 4－5　土壌の物理分析成績

タナブランゲ(2)

層位	仮比重	pF1.5 三相分布 %			全孔隙%	飽水度 pF1.5%	有効水分	透水係数
		固相	液相	気相				
1	1.264	52.5	40.0	7.5	47.5	84.3	6.7	2.9×10^{-3}
2	1.295	49.8	41.8	8.5	50.2	83.2	5.5	1.6×10^{-2}

化学分析成績では第1層の微砂質層が他所から流れ込んだ別の母材によるものらしく，N−KCl 浸出のカルシウム，マグネシウム含量が著しく低くなっており，有効態リン酸も少ない。第2層はタナブランゲ(1)に似ているが，カルシウム含量は約半分であって，化学的にやや劣るようである。

管理対策としては土層の内部排水を良くするためにこの区域の中に要所に明きょを掘って水を早く流すことが必要である。南側を流れる川に水を落せばよいのであるが，その間に桑園内の幹線道路があり，この踏み固めによって土層の内部排水が遮断されることもありうるので，道路の下を通す水の通路が必要なように考えられる。むしろ川に接した物理性の良い地帯の桑園の改善を行なう方が管理労力としても効果的と思われる。

Table 4－6　土壌の化学分析成績　　タナブランゲ (2)

層位	pH		置換酸度 Y_1	N−KCl 可溶 m.e		全窒素 mg %	リン酸		灼熱損失%
	H_2O	KCl		CaO	MgO		有効態 mg	吸収係数	
1		4.4	2.6	6.7	2.8	18.1	3.2	1910	2.55
2		5.0	0.9	18.0	4.3	47.5	4.2		1.73

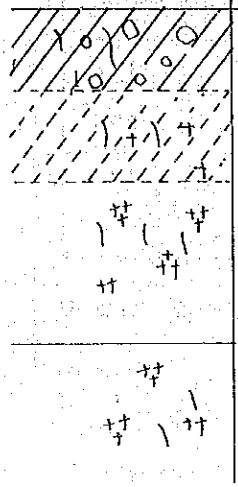
5）ルバンゲ桑園

タナブランゲ桑園から南下して，洪積台地に上ったところに位置しており，東および北に向ってゆるく傾斜している。

土層の中に石灰岩質のれきが多く，暗色のれきとまじりあっている。断面の特徴は Table 5－1 のとおりで，第3層までが埴土，第4層が砂土ということから，その直上部に滞水しやすい条件が出来るものと思われる。第2・第3層は埴土といっても埴壌土に近い微砂質のもので風化れきの斑紋や，れきの表面に Mn が沈着して出来た斑紋がみられる。また第4層には鉄の斑紋があって台地上にありながらも，一時的に土層が湿潤になるものと考えられる。

Table 5－1　土壌断面の特徴

ルパングー

土壌断面	層位深さcm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	黒褐色	埴土	なし	塊状	24 mm	細含む
	20cm						
	2	にぶい黄褐色	微砂質埴土	風化れき斑紋	堅果状	22 mm	細あり
	37						
	3	にぶい黄褐色 黄褐色	微砂質埴土	れきの表面にMn斑	堅果状	20	細あり
	85						
	4	褐色 黄褐色	砂土	れきの表面にFe斑	細粒状	24	細あり
	100+						

土壌の物理性をTable 5－2 に示したが，仮比重が約 1 ～ 12 の間にあり，固相・液相・気相のバランスも良く，有効水分も平均 13 %程度で良好である。飽水度も予想より小さく，適潤の範囲に入るようで，物理的な問題はない。

Table5－3 に示した化学性もタナブランゲ(1)に似ており，酸度が小さく，著しくカルシウムに富んでいる。しかしマグネシウム含量が絶対量は足りているものの相対的に含量が低いことが気になる点である。それと，共通していることであるが全窒素の水準が低い。

Table 5－2　土壌の物理分析成績

ルパングー

層位	仮比重	pF 1.5　三相分布 %			全孔隙%	飽水度 pF 1.5 %	有効水分%	透水係数
		固相	液相	気相				
1	0.977	35.5	41.1	23.4	64.5	63.7	10.9	6.7×10^{-3}
2	1.187	42.7	39.7	17.6	57.3	69.3	10.9	6.2×10^{-3}
3	1.125	40.7	43.2	16.1	59.3	72.8	16.5	3.4×10^{-3}
4	1.069	38.2	39.0	22.8	61.8	63.1	13.8	2.1×10^{-2}

Table 5－3　土壌の化学分析成績

ルパンゲ

層位	pH		置換	N-KCl可溶m.e		全窒素mg%	リン酸		灼熱損失%
	H_2O	KCl	酸度Y_1	Cao	Mgo		有効態mg	吸収係数	
1	6.4	6.7	0.7	44.6	1.1	14.9	10.9		1.16
2	8.4	7.6	0.7	43.9	1.9	69.2	6.7		0.93
3	8.2	7.2	0.8	49.1	2.4	27.9	11.3		1.33
4	8.2	6.5	0.3	41.5	3.2	0	3.13		—

今後の対策としては窒素肥料を主体にした施肥の充実である。カルシウムとのバランスがやや悪いが，マグネシウム欠乏になることはまずないであろう。

6）ウ　ギ　桑　園

タナブランゲの東の方向でテムベ湖の南側の，地図上では湿地帯の記号が入っている川岸の桑園である。川は土砂の堆積物を削って流れており，河原を持たない。山岳地帯での侵食の激しさを思わせるものがある。

土壌断面の特徴はTable 6－1 に示したが，沖積土特有の土性の極端に異る互層はみられず表土の土性が埴壌土であるのに対し，第2・第3層は微砂質埴土で，物理性は良いようである。また第3層は古い表土の埋没したものと思われ，腐植がやや多い。

Table 6－1　土壌断面の特徴

ウギ

土壌断面	層位 深さcm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	黒褐色	埴壌土	なし	軟粒状	17 mm	細，含む（5%）
	20cm						
	2	暗褐色	微砂質壌土	なし	堅果状	25	細，含む（3%）
	78						
	3	暗褐色	微砂質壌土	なし	堅果状	23	細，あり（1%）
	100						

土壌の物理性をTable 6－2 に示した。仮比重は1～1.2 の間にあり，問題はないが，三

相分布の中の気相率が第１・第２層でやや小さい。したがって飽水度はやや高く，冠水することが多い環境を反映している。また有効水分は著しく多く火山灰土に近い値を持っている。

化学分析の結果はTable6－3 のとおりで，川がタナブラング方面から流れてきているので，タナブランゲ，ルバンゲ土壌と類似した結果となっている。したがってカルシウムに富んでい

Table 6－2　土壌の物理分析成績

ウギ

層位	仮比重	pF 1.5　三相分布　%			全孔隙%	飽水度	有効水分%	透水係数
		固相	液相	気相		pF 1.5 %		
1	1.006	36.8	49.6	13.6	63.2	78.5	22.8	3.0×10^{-3}
2	1.149	42.2	43.7	14.1	57.8	75.6	16.7	3.0×10^{-3}
3	1.169	34.4	36.7	28.9	65.6	55.9	16.7	1.1×10^{-2}

て酸度が低く，有効態リン酸もかなり含まれている。また洪水があるためか，全窒素の値が第１，第２層（ 78 cm まで）とやや高く，総合的にすぐれた桑園の一つである。しかし表層の有効態リン酸がやや少ない。

Table 6－3　土壌の化学分析成績

ウギ

層位	pH		置換	N－KCl 可溶 m.e		全窒素mg%	リン酸		灼熱損失　%
	H_2O	KCl	酸度Y_1	Cao	Mgo		有効態mg	吸収係数	
1		6.8	0.7	33.4	2.9	64.0	0.6		2.63
2		6.8	1.0	31.3	2.9	80.9	6.9		2.58
3		6.8	0.7	33.2	2.6	42.3	8.1		2.32

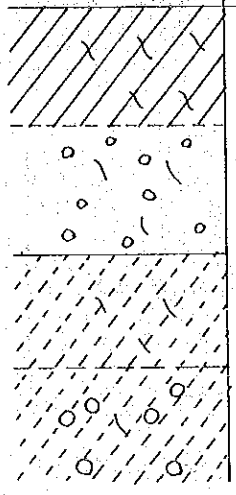
問題点は雨期に冠水することであるが，それに対する対策はない。ただ施肥の時期については，冠水によって流失させない考慮を要する。

7）ワニヨ桑園

タナブランゲから北上してシドラップ県を越えた地域のテムペ湖の西側にある川沿いの桑園である。Table 7－1 に示すように土性は埴壌土と砂土の互層でコンシステンシーが小さい。互層でありながら斑鉄がみられないのは，川岸であるため横方向への排水も進むためであろう。物理的には良好な土壌と思われる。川の状況としてはウギよりも流れが早く水が引きやすいようで，砂土の層が厚いのはそのためであろう。

Table 7－1　土壌断面の特徴

ワニヨ

土壌断面	層位 深さ cm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	黒褐色	埴壌土	なし	粗粒状	13 ㎜	細あり3％
	24 cm						
	2	褐色	砂土	なし	無構造	11	細あり1％
	58						
	3	暗褐色	埴壌土	なし	堅果状	19	細あり1％
	78						
	4	暗褐色	砂土	なし	無構造	11	乏し

物理的性質はTable 7－2 に示したが，仮比重が大きく固相率が大きいことが特徴的である。砂質の土層は充塡が起りやすいが，特に第2層は常識的には不良土となるはずであるが，粗孔隙や飽水度には問題がなく，また有効水分は第4層とともに著しく豊かである。このように仮比重が大きいものの，例外的に物理性は良いようである。

化学分析成績については，この地方の他の調査例にくらべるとカルシウム，マグネシウムが減少しているが，酸度は小さい。これは土壌が砂質であることにもよる。第2，第4層は特に

Table 7－2　土壌の物理分析成績

ワニヨ

層位	仮比重	pF 1.5　三相分布　％			全孔隙％	飽水度 pF 1.5 ％	有効水分％	透水係数
		固相	液相	気相				
1	1.246	46.2	38.4	15.4	53.8	71.4	14.7	8.4×10^{-4}
2	1.366	48.3	37.5	14.2	51.7	72.5	24.6	1.2×10^{-3}
3	1.331	48.4	40.4	11.2	51.6	78.3	11.4	1.9×10^{-3}
4	1.322	49.6	32.8	17.5	50.4	65.3	20.4	2.7×10^{-2}

であることにもよる。第2，第4層は特に砂質であるために第1，第3層に比べても半減している。そして全窒素量が著しく小さい。当然窒素を中心とした施肥が必要であるが，窒素肥料を施すと土壌が酸性化するので，特にカルシウム含量が小さいだけに雨期を考慮した施肥設計を行ない。窒素を流亡させた上に土壌の酸性化をすすめることのないよう注意しなければなら

Table 7－3　土壌の化学分析成績

カニヨ

層位	pH		置換酸度 Y_1	N−KCl 可溶 m.e		全窒素%	リン酸		灼熱損失%
	H_2O	KCl		CaO	MgO		有効態 mg	吸収係数	
1	7.0	5.8	0.3	15.1	4.2	0.8	14.2		2.21
2	5.8	5.5	0.3	7.6	2.4	13.6	14.1		1.08
3	5.0	5.4	0.3	15.1	4.0	28.5	18.7		1.60
4	4.8	5.4	0.3	9.8	2.4	2.3	12.0		0.93

ない。

第2層が砂土であるため肥料の流亡は起りやすいであろう。

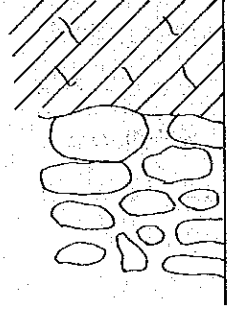
8）マセッペ桑園

ワニヨ桑園からさらに北上した台地上にあり，周辺は草地が西の山すそまで拡がっている。桑園の西側の谷に向って緩傾斜する形となっているが，所々に葉の黄化がみられ，小溝侵食を受けた場所では母岩が頭を出しているところもある。その黄化葉の原因が干害のためであると推定されたので，表土から約 40 cm 掘り下げて原因を調査することにした。

土壌断面の特徴をTable 8－1 として示したが，僅か 12cm の深さに母岩層が現れ，30cm しか掘らなかった。母岩の割れ目にはチョコレート色に風化した母材が網の目のように入っており，母岩自身も砕けやすく風化していた。グリーンタフ様の凝灰岩と思われたがハンディペネトロメーターが貫入できる程母岩の風化は進んでいた。このため桑の根も部分的には入りうるものと思われるが絶対量としての分布は少ないから干害にかかりやすいのは当然で，桑葉が黄化した理由は水不足が主なもので，チョコレート色の母材ということから一部には重金属の過剰吸収ということも考慮しなければならない。

Table 8－1　土壌断面の特徴

マセッペ

土壌断面	層位 深さ cm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	黒褐色	埴壌土	なし	粗粒状	18 mm	細あり 2 %
	12cm						
	2	灰色	風化した母岩（凝灰岩質）	なし		29	なし
	30cm+						

土壌の物理性についてはTable 8－2 に示したが，第２層の風化した母岩層も採土が出来た。その程度には風化が進んでいるからブルドーザのような機械力を使えば，かなりの深さまで耕起できるであろう。未熟な残積土であるから仮比重は重く固相率は 50 ～ 55 ％で耕作不適というには至らない。気相率（二粗孔隙）は第２層で 10 ％以下であり，根の伸長は困難であろう。飽水度が示す湿潤度も第２層で高く，湿害の可能性を示している。有効水分は第１層で約９％であるから熟畑化すれば普通の保水力は持つであろう。

Table 8－2　土壌の物理分析成績

マセッペ

層位	仮比重	pF 1.5 三相分布 ％			全孔隙％	飽水度 pF 1.5 ％	有効水分％	透水係数
		固相	液相	気相				
1	1.464	51.1	38.0	10.9	48.9	77.7	9.2	5.1×10^{-4}
2	1.559	54.7	37.9	7.4	45.3	83.7	5.1	5.9×10^{-4}

化学分析の結果を Table 8-3 に示したが，酸度は小さく，カルシウム，マグネシウムも多い。しかし窒素とリン酸はやや少ない。

管理対策は耕土層を深くすることが最大の目標である。

Table 8－3　土壌の化学分析成績

マセッペ

層位	pH		置換酸度 Y_1	N－KCl 可溶m.e		全窒素mg％	リン酸		灼熱損失％
	H_2O	KCl		CaO	MgO		有効態mg	吸収係数	
1	6.8	5.3	0.4	14.5	4.7	62.0	2.8		1.73
2	7.2	5.7	0.3	17.2	4.8	19.0	1.2		1.10

9）バラッカ桑園

エンレカンの山間地に開かれた桑園であるが，この附近一帯に石灰岩のドームが散在しており，その周辺に泥岩・頁岩の風化物が極めて粘土質の土壌を作っている。この桑園の上部は傾斜が急で，一部に母岩が露出しており，その周辺の桑は伸びが悪い。また凹地になっている場所は，表土まで湿潤で桑の生育が悪く，湿潤を好むロンタラの木が生えていた。また畦の立て方が傾斜方向であるため侵食を助長するものと思われる。桑園の最も低い場所は傾斜も５° 程度であるが，ここでは桑がよく茂っていた。

土壌断面の特徴をTable 9－1に示す。土色は黄褐色が基調になっているが，第2層に部分的に見られたグライ斑は第3層と第4層にかけて次第に量が多くなり，モザイク状になっている。土性は全層埴土で水分を多く含み，粘着性が強い。グライ斑が見られるにもかかわらず桑根は少いながら全層に分布していた。

Table 9－1　土壌断面の特徴　　バラッカ

土壌断面	層位 深さcm	土色	土性	斑紋	構造	コンシステンシー	根系
	1	明黄褐色	埴土	なし	塊状	16 mm	細小とむ　7％
	20 cm						
	2	明黄褐色 にぶい黄褐色	埴土	グライ斑 1％	塊状	11	細～中含む　5％
	33						
	3	黄褐色 褐灰色	埴土	グライ斑 5％	塊状	15	細～中含む　3％
	70						
	4	黄褐色 褐灰色	埴土	グライ斑 5％	塊状	17	細あり　1％
	100+						

Table 9－2 に示した物理性では仮比重，固相率が極端に大きいわけではないが，液相率が過大で第2層以下は根が伸長できないような粗孔隙（気相率）しか存在しない。断面調査で僅かながら根が観察されたことが不思議な程である。また透水性が極端に悪く，とくに第3層は不透水といってよい。

Table 9－2　土壌の物理分析成績　　バラッカ

層位	仮比重	pF1.5 三相分布 %			全孔隙%	飽水度 pF 1.5%	有効水分%	透水係数
		固相	液相	気相				
1	1.253	46.5	34.7	18.8	53.5	64.9	6.1	2.5×10^{-2}
2	1.340	49.5	43.7	6.8	50.5	86.5	5.2	3.4×10^{-7}
3	1.410	52.8	45.8	1.4	47.2	97.1	3.6	8.5×10^{-9}
4	1.448	53.8	42.7	3.4	46.1	92.7	6.6	3.3×10^{-6}

10） ス ズ 桑 園

バラッカ桑園と状況は似ているが，西側数百メートルのところに石灰岩壁がそびえている。そのふもとから緩傾斜がつづいており，道路に近い場所に桑園がある。凹地は湿性植物が生え，桑の枯死株が多い。深さ 50 cm の簡易調査を行った。

土壌断面の特徴をTable 10−1 に示したが，バラッカ同様全層埴土で，第2層・第3層にはグライ斑が現れている。また，全層に巻貝が散在していたが比較的新らしいようで，かっては湿地帯であったと思われる。バラッカより赤味の強い土色である他は類似した点が多い。生育はバラッカと似ているがやや劣るようである。

Table 10−1 土壌断面の特徴

スズ

土壌断面	層位 深さcm	土色	土性	斑紋	構造	コンシステンシー	根系
12cm	1	褐色	埴土	なし	塊状状	19 mm	細あり 2%
	20cm						
	2	褐色 褐灰色	埴土	グライ斑 3%	塊状	18	細あり 1%
	40						
	3	にぶい褐色	埴土	グライ斑 7%	塊状	11	なし
	50 +	褐色					

土壌の物理性をTable 10−2 に示すが，バラッカと似ており，しかも，第1層から物理性が悪く，表土の侵食を推定させる。仮比重はむしろ良好な範囲にあるが液相率が異常に高い点はバラッカと同様である。有効水分は少ない。土層を乾かしてクラックが入るようにしないと，この状態は変らないであろう。マルチもしくは高畦密植ということも一つの方法であろう。

Table 10−2 土壌の物理分析成績

スズ

層位	仮比重	pF 1.5 三相分布 %			全孔隙%	飽水度 pF 1.5 %	有効水分%	透水係数
		固相	液相	気相				
1	1,065	44.2	50.4	5.4	55.8	90.4	5.7	6.2×10^{-5}
2	1,102	46.2	52.3	1.5	53.8	93.5	5.4	2.5×10^{-8}
3	1,010	41.9	58.4	(−0.2)	58.1	(100.5)	5.6	1.4×10^{-8}

2 結　　び

南スラウェシ州において行った桑園土壌調査の結果から，地域的に3つのグループに分けることができる。

一つは，パカトー，ビリビリ，マリノーのグループで，火山起源の岩石を母材としており，浮石あるいは凝灰岩が含まれるようで，標高が高まるにつれて火山灰が加わっているようである。低地のパカトーでは物理性は不良とは言えないが，やや湿性の土壌である。化学的にはマグネシウムの含量が高く，欠点としては全窒素の不足をあげることができる。

第2のグループはテムペ湖周辺のものであるが，西側の山地が石灰岩地帯であるため，いずれも石灰含量は高い。しかし，洪積のテラスから低地を移った場所にあるタナブランゲでは粘土質の土壌で粗孔隙が少なく，水分供給力が小さい。これに対して，洪積テラス上のルパングや，川岸のウギ，ワニヨでは物理性が良くなっている。したがってこの地域の桑園造成に関しては川に近い場所とか，高台を選ぶかすることが安全といえよう。またテムペ湖北西部の山麓地帯には広い草原が形成されているが，この地域は土壌侵食によって表土が失なわれ，母岩が浅く現われるため，水不足，あるいは排水不良のため耕作に適さないと考えられる。しかし風化した母岩は比較的砕けやすいので，大型機械で耕起し，土層を深くすれば耕作が可能と考えられる。

第3のエンレカングループは，サダン河の濁流に象徴されるように，非常に粘土質で内部排水が悪いため，桑の生育が良くないと言える。土層としては深いようであるから，高うね式にするとか，マルチによって深い土層の水分を利用するとかの方法で表土を利用する対策が必要であろう。

全体としてカルシウム，マグネシウムの含量が多く，有効態リン酸もかなり含まれている一方で，窒素水準が低いので，窒素肥料を中心とした施肥技術の普及が望まれる。それと並んで有機物含量が低く（灼熱損失ビリビリを除き4％以下），安定した地力を持つとはいえない。物理性の悪い場所では特に必要であるが，しきわら，しき草のような形の有機物を導入することが望まれる。

なお，この調査を遂行するにあたり，計画の立案・調査日程の調整・分析作業に必要な機器の調達について，派遣専門家の森信行リーダーをはじめ，山本賢・富永勝廣氏に多大のご援助をいただいた。また，西昇一郎・井原音重・阿部芳彦各氏には，実験器具・薬品などの貸・供与をいただいた。調査研究がとどこおりなく進んだことは，各位のご厚情のたまものとして感謝申し上げる。