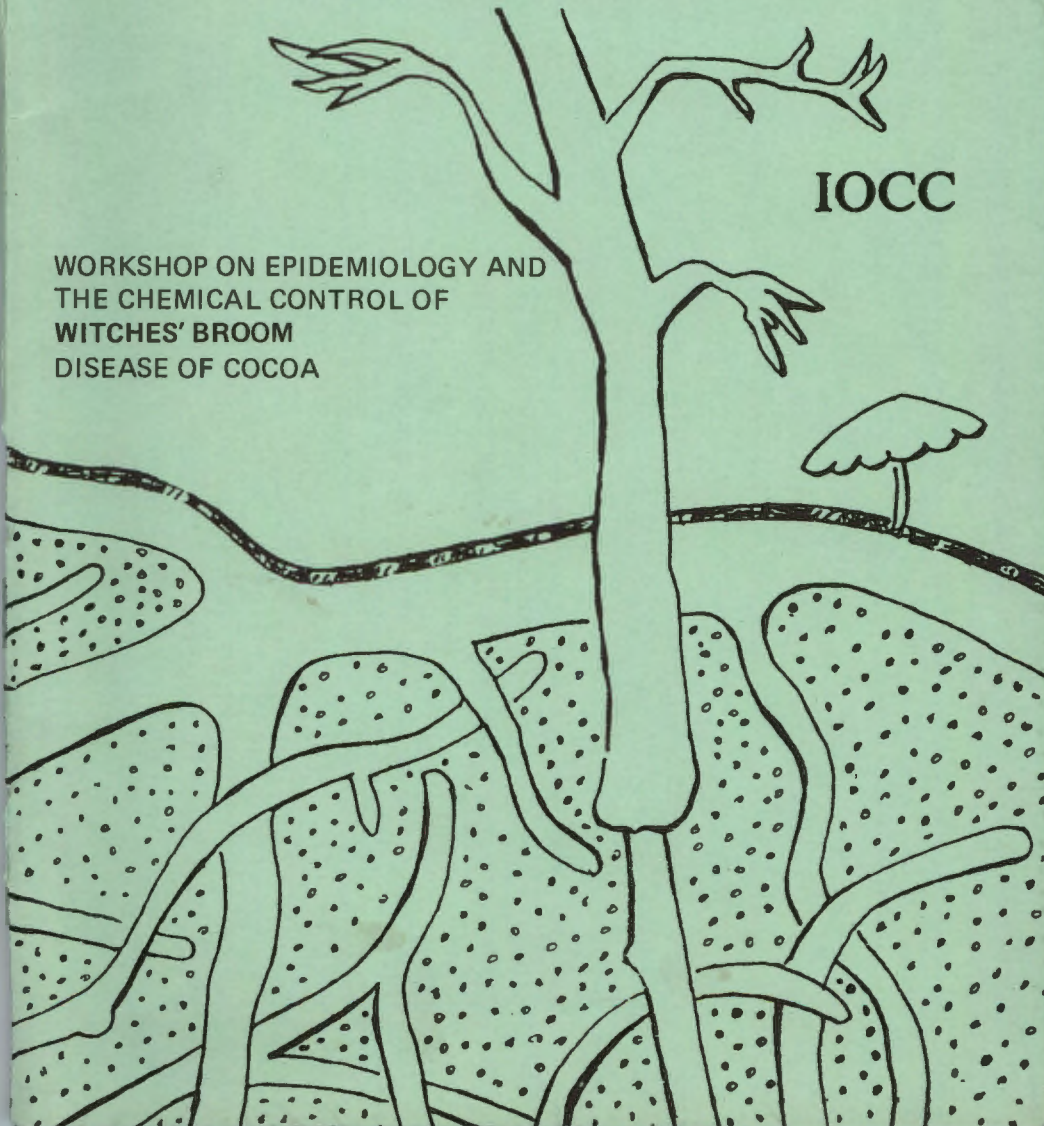


MANAGING WITCHES' BROOM DISEASE OF COCOA

REPORT OF 3rd WORKSHOP
OF INTERNATIONAL WITCHES' BROOM PROJECT
17 – 18 June 1986, Manizales, Columbia.

IOCC

WORKSHOP ON EPIDEMIOLOGY AND
THE CHEMICAL CONTROL OF
WITCHES' BROOM
DISEASE OF COCOA



INTERNATIONAL WITCHES' BROOM PROJECT

(IWBP)

IWBP is an international project funded by chocolate manufacturers in Europe and North America with a number of International Agencies and with the governments of 4 cocoa producing countries. It proposes to conduct fundamental research at a total of 13 centres in Europe, Latin America and the United States aimed at a reduction of the devastation caused by Witches' Broom disease of cocoa due to the pathogen Crinipellis perniciosa (Stahel) Singer.

International Office of Cocoa and Chocolate
Office International du Cacao et du Chocolat
Avenue de Cortenbergh, 172
BRUSSELS, 1040
Belgium

REPORT OF THE 3RD IWBP WORKSHOP

7th - 12th July 1986

Held at: Granja Luker
 Chocolateria Luker
 Santagueda
 Manizales
 Colombia

Host: ICA/Chocolateria Luker/IOCC

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Edited by: R.A. Lass and S.A. Rudgard

C O N T E N T S

	<u>Page</u>
1. Introduction to IWBP	1
2. Narrative Report of the 3rd IWBP Workshop	
2.1 Opening Session	4
2.2 Discussion on Objectives of IWBP	6
3. 'Comparative Epidemiology Experiment'	
3.1 Status of the 'Comparative Epidemiology Experiment' as of 30th June 1986	6
3.2 Discussion on the methodology of the 'Comparative Epidemiology Experiment'	7
3.3 Preparation of Protocol IA of 'Comparative Epidemiology Experiment'	8
3.4 Text of Protocol IB of the 'Comparative Epidemiology Experiment'	12
3.5 Action to be taken on 'Comparative Epidemiology Experiment'	15
4. 'Inoculum and/or Disease Gradient Experiments'	
4.1 Introduction	16
4.2 Guidelines for conduct of 'Disease Gradient Experiment'	16
5. Experimentation on Chemical Evaluation	
5.1 Introduction	18
5.2 'Guidelines for Chemical Evaluation Experiments'	18
5.2.1 Experiments Under Controlled Conditions	18
5.2.2 Field Tests	20
6. Phytosanitation Experiments	21
7. Acknowledgements	22
8. Date for the 4th IWBP Workshop	22
9. Date for the next meeting of the Project Scientific Committee	22

1. INTRODUCTION TO IWBP

Almost one-third of the world supply of cocoa is produced in the Americas but in many countries maximum production is severely curtailed by Witches' Broom (WB) disease. It occurs in the upper Amazon region of Brazil, in Bolivia, Colombia, Ecuador, Grenada, Guyana, Peru, Surinam, Trinidad and Venezuela. This widespread distribution also places at risk other areas where it is not yet present, such as Bahia, Brazil - the second largest concentration of cocoa plantings in the world - or proposed new plantings of cocoa such as in Amazonia, Brazil. In the Brazilian Amazon pod losses have reached 95 per cent on occasions, and almost all infected pods are a total economic loss. Moreover, there is NO assurance that its absence from cocoa producing countries outside the Americas will continue.

These features make a co-ordinated, multi-centre, multi-national research program on WB a top priority and so a project proposal was formulated by scientists in countries where the disease is important. The pathogen (Crinipellis perniciosa) causes overdevelopment of cocoa meristematic tissues leading to hypertrophied and proliferated growth, to give the characteristic Witches Brooms (Escoba de Bruja/Vassoura-de-Bruja/Balai de Sorciere). It also infects many pods. Infected pods fail to ripen and the beans either become liquified or adhere to the pod husk and are commercially valueless. In view of this serious threat, the International Office of Cocoa and Chocolate (IOCC) with some other International Donor Agencies are funding a US \$ 700,000 multinational research project over a period of about 5 years from 1st July 1985. The objective of the Project is to develop practical, reliable economic methods of managing the disease in a number of cocoa growing environments.

In early July 1985 participants in IWBP met at the 1st IWBP Workshop to develop a major research programme on the epidemiology of Crinipellis perniciosa and the decisions on this were published in October 1985 in the 'Report of the 1st IWBP Workshop'. The experiments to investigate the epidemiology are now under way in seven sites in four countries.

It was considered important to start to consider the evaluation of candidate chemicals for disease control and a preliminary discussion was held at the 2nd IWBP Workshop held in Wageningen, Holland in October 1985. This discussion was published in the 'Report of the 2nd IWBP Workshop'.

Copies of both these reports are available free of charge from Dr A Van Tulder, IOCC, Avenue de Cortenbergh 172, Brussels, 1040, Belgium.

2. NARRATIVE REPORT OF 3RD IWBP WORKSHOP

2.1 OPENING SESSION

Participants assembled at the Granja Luker owned by Chocolateria Luker on Sunday 5th and Monday 6th July 1986. The names, addresses, telephone and telex numbers (where applicable) of all the participants are included as Appendix A to this report. The Workshop was hosted by Chocolateria Luker who most generously provided free accommodation to all the participants. IOCC wish to record their very great appreciation to the management of Chocolateria Luker for their hospitality.

The 3rd IWBP Workshop started at 08.00 on Monday 6th July 1986 with a welcome from the Research Manager for Industrial Crops in ICA (- Pablo Buritica), Director for Region 9 of ICA (Rafael Estrada), Manager of Granja Luker (Alberto Agudelo Munoz), on behalf of Chocolateria Luker and the Executive Chairman of IWBP on behalf of IOCC. The last address was as follows:

"Rafael Estrada Director, Region 9, Instituto Colombiano Agropecuario representatives of our hosts Chocolateria Luker S.A., fellow participants in the International Witches' Broom Project and observers.

I use the words 'Observer' deliberately because we have present here at Granja Luker representatives of a number of bodies who are not as yet fully participating in IWBP. In this connection I mention:

- Mrs Jennifer Andell from Grenada (whose attendance is funded by Canadian International Development Association (CIDA);
- Humberto and Lilien Reyes of FONAIAP, Venezuela whose attendance is funded by FONAIAP;

The 1st IWBP Workshop was held at INIAP Pichilingue exactly twelve months ago and we developed the Protocol for the 'Comparative Epidemiology Experiment' which is now in progress at seven sites. This is most satisfactory. The data recording must now continue reliably and consistently and this week we must develop a procedure for the comparative analysis of the considerable quantity of data which is being collected as well as discussing any problems which have arisen in the operation of the epidemiology experiment.

The 2nd IWBP Workshop was held in Wageningen, Holland in October 1985 and was a preliminary discussion on the prospects for chemical control of the pathogen. Clearly chemical control could play an important role in any disease management system and so we must therefore also develop a protocol for chemical evaluation during the course of this week.

The objective of IWBP is to manage witches' broom disease and so we must evaluate any techniques presently employed and perhaps propose phytosanitation experiments as well as giving consideration to the need for other experiments. We will also be discussing these this week and developing any necessary protocols. We have a busy programme ahead of us. We have most pleasant surroundings in which to work and I would like to thank Luker Chocolates most warmly for their kind hospitality.

May I close by extending fraternal greetings from the President of the International Office of Cocoa and Chocolate to all participants in the 3rd Workshop of the International Witches' Broom Project and extend to you his good wishes for a most successful and pleasant Workshop leading to useful results towards the achievement of the IWBP objective of 'Managing Witches' Broom Disease of Cocoa'.

The Agenda for the 3rd IWBP Workshop was adopted and is included as Appendix B to this report. The Objectives of the 3rd IWBP Workshop were fully discussed, agreed and then adopted as follows:

1. To reaffirm objectives of the 'Comparative Epidemiology Experiment';

2. To discuss methodology of data collection on the epidemiological parameters in Year 1 of the 'Comparative Epidemiology Experiment' and refine this methodology for Year 2;
3. To discuss data analysis by site for the 'Comparative Epidemiology Experiment' and develop a protocol for the data analyses;
4. To discuss disease gradient studies for Witches' Broom at IWPB sites, and if appropriate, develop a protocol;
5. To discuss and develop protocol(s) for chemical evaluation as part of IWPB;
6. To discuss phytosanitation experiments for the management of Witches' Broom as part of IWPB;
7. To develop the procedures for preparation of the IWPB Annual Report;
8. To report on scientific activities that complement IWPB;

2.2 OBJECTIVES OF THE INTERNATIONAL WITCHES' BROOM PROJECT

These were fully discussed once more during the 3rd Workshop and reaffirmed with the following amendment. It was agreed that the following additional broad study should be added to the IWPB objectives as agreed at the 1st Workshop of IWPB at Pichilingue in July 1985.

- "c. To examine and evaluate phytosanitary practices and their impact on Witches' Broom disease".

[Note: the wording agreed above should be inserted after line 3 on page 3 of the Report of 1st Workshop of the International Witches' Broom Project.]

3. 'COMPARATIVE EPIDEMIOLOGY EXPERIMENT'

3.1 STATUS OF THE 'COMPARATIVE EPIDEMIOLOGY EXPERIMENT' AS OF 30TH JUNE 1986

Brazil

Research is coordinated in Belem by Dr Teklu Andebrhan, and the two operators are: Altamira, Joao de Cassia do Bomfim Costa and Tome Acu, Ausier Luiz Pacheco de Carvalho. Tecnico Emanuel Pantoja has assisted with data recording in Altamira.

Colombia

Research is coordinated in Manizales by Fabio Aranzazu Hernandez, and the two operators are; Llanos, Carlos Coral and Santagueda, Luiz Carlos Villegas.

Ecuador

Research is coordinated at Pichilingue by Dr Alan Maddison, assisted by Ing Jaime Aragundi and Ing Guido Solorzano, and the operators for the two sites are Carlos Moreira and Gustavo Macias. Agr. Neira has also assisted in data recording.

Trinidad

Research is coordinated by Dr T N Sreenivasan, and the operator at Santa Cruz is Steve Mohan.

Data sets available for Comparative Epidemiology Experiment:

Country	1985												1986												1987											
Site	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A				
<u>Brazil</u>																																				
Altamira								+																												
Tome Acu								+																												
Ouro Preto*																																				
<u>Colombia</u>																																				
Llanos								+																												
Manizales								+																												
<u>Ecuador</u>																																				
Station								+																												
Farm																																				
<u>Trinidad</u>																																				
Santa Cruz																																				
<u>Venezuela</u>																																				
Irapa*																																				

* Units yet to start recording as of 5th July 1986

+ Installation of meteorology equipment

3.2 DISCUSSION ON THE METHODOLOGY OF THE 'COMPARATIVE EPIDEMIOLOGY EXPERIMENT'

There were now several of months data available from 6 of the 8 experimental sites. The Protocol for the methodology for this experiment is fully described in pages 14-23 of the 'Report of 1st Workshop of IWBP' published by IOCC in August 1985 and was based on the agreement of all participants at that time. This Protocol is hence forward called 'IWBP PROTOCOL I'.

The methods of collection of the data, the precise meaning of the actual terminology as outlined in the above Protocol were discussed at considerable length during the course of the present workshop. Some changes and clarifications were agreed and these are reproduced below. The proposals for analysis of the data arising from the experimental work were discussed and agreed. The analyses which will be carried out are now included in Protocol IA. These amendments were accepted for incorporation in Protocol I which will henceforward called 'IWBP PROTOCOL IA'.

It was further agreed that 2 or more reference sites should be established in areas where the disease is absent and that the meteorological parameters should be measured for these sites. This data would be collected following Protocol IB which is reproduced in paragraph 3.4.

3.3 PROTOCOL 1A OF THE 'COMPARATIVE EPIDEMIOLOGY EXPERIMENT'

The following amendments to IWPB Protocol I were adopted:

Amendment 1 Delete number prefix 5.5 throughout Protocol 1 of Comparative Experiments, thus greatly simplifying the numbering of the paragraphs.

Amendment 2 Insert: new item at end of 1.b:

" 10. Cultural practices. "

Amendment 3 After "observed" in last line of paragraph 2.a. insert:

" and labelled with date (week number) and the initial symptom. "

Amendment 4 After "data sets" in paragraph 2.c. insert:

" and five additional 1m lengths in the branches of the tree. "

Amendment 5 Insert new items in paragraph 2.c.:

- " 5. Number of fruits newly recorded as chirimoyas.
These are not removed.
6. Number of pods with 'green islands'. "

Amendment 6 After "recorded" in last line of paragraph 2.c. insert:

" all other diseased and ripe pods on the trees in the plot are removed and counted every 2 weeks. The height of each pod to the nearest 50 cm is recorded on the ten sample trees. "

Amendment 7 Delete items i), ii) and iii) of paragraph 2.d.
Replace with the following:

- " i) Suspended Vegetative Brooms
a. On the samples of brooms hung in the tree in Year 1. When these brooms fall they are not replaced.
b. On each numbered tree a new representative sample of 10 numbered dead brooms hung at the top of the canopy and another sample of 10 numbered, dead brooms hung in the middle of the canopy. These brooms are obtained within the plantation. If marked brooms fall they are re-hung if possible or, if not, are replaced.

ii) Attached Brooms

Attached brooms formed in Year 1 up to a maximum of 10 brooms per tree within the marked units.

iii) Brooms on Ground

- a. On brooms placed beneath each numbered tree in Year 1.

- b. A new sample of 10 numbered dead fallen brooms placed beneath each tree in Year 2. (At some sites only, an additional sample of 10 numbered pruned brooms).

iv) Cushions

- a. On a maximum of 20 infected cushions per tree identified in Year 1. The remaining cushions infected in Year 1 are retained. Fallen infected material is not replaced.
- b. On each marked tree, where they are present 20 infected cushions distributed on the tree. In situations where they are not present, then on trees outside the plot, 20 per tree where available. If this option is not possible, on cushion brooms obtained from outside the site and attached to marked trees to a maximum of 20 per tree.

Re number existing items (iv) and (v) as items (v) and (vi) respectively.

Amendment 8 Insert at end of new paragraph 2.d. v):

" fresh samples of pods are added in Year 2. "

Amendment 9 Insert new sentence at end of paragraph 2.d:

" If an object is lost (L) or replaced (R) it should be noted in the appropriate position on the data sheets in the week in which it occurs. "

Amendment 10 Insert at end of paragraph 2.e:

" Brooms have been or should be removed (+) in accord with the following scheme:

<u>Location</u>	<u>Plot</u> (10 trees)		<u>Site</u>		<u>Plantation</u>	
	Yr 1	Yr 2	Yr 1	Yr 2	Yr 1	Yr 2
Ecuador - Station*	-	+	-	+	-	+
Farm	-	-	-	-	-	-
Brazil - Tome Acu	+	-	+	-	+	N/A
Altamira	+	-	+	-	+	N/A
Ouro Preto	+	-	+	-	+	N/A
Colombia - Manizales	+	-	+	-	-	-
- Llanos	+	-	+	-	+	+
Trinidad	+	-	+	-	-	-
Venezuela	+	-	+	-	+	+
Recommended practice**	+	-	-	-	-	-

* There will be a third year of data from this site.

** Divergences allowed, based on local management practices.

/A Decision not necessary at these locations (plot and site only)

Amendment 11 Delete list of equipment in 2.f.iv). Replace with the following:

" Casella thermohygrograph
Weighing bucket raingauge
Belfort dew recorder
Maximum and minimum thermometer
Sling psychrometer "

Amendment 12 Delete "aspirated" in text of 2.f. ii), and insert:
" sling "

Amendment 13 Delete last two lines of paragraph 2.f. iii). Replace with the following:

" Rain: For each event, start, finish, duration, amount.
Wetness: For each event, start and finish of any deflection, maximum % deflection recorded, duration of 100% deflection and cause of event (rain and/or dew).
Day of reference is day event begins. "

Amendment 14 Insert new item after paragraph 2.f.:

" g. Sampling weeks begin on Mondays and end on Sundays. For newly established sites the observations made every two weeks should start in an odd-numbered week. "

Amendment 15 Insert new item after 5.5.2.g.

" h. Calibration and Maintenance of Meteorological Equipment

- i) When charts are changed on all machines, it should be checked that:
 1. Pens are writing properly;
 2. Chart movement is correct; or any time lag noted;
 3. Pen is properly in contact with new chart.
 4. Use pen to mark vertically the end of old chart record and beginning of the new chart.
- ii) At the end of every four weeks, temperature and humidity elements of both the dew recorder and thermohygrograph should be checked or calibrated by psychrometer (sling or other). If registering incorrectly, the temperature elements should be calibrated to an air temperature of approximately 25°C to give least error at 20° and 30°C limits.

If the humidity elements have not been recording maximum deflection at saturation then should be wetted thoroughly, left for minutes and set to 95%. Once they have equilibrated with ambient conditions, they should be checked at a low humidity around midday and adjusted if necessary. Hair

elements should be cleaned with a soft paint brush and distilled water every 6 months. Strings on dew recorders should be changed every 6 months. "

Amendment 16 Delete existing paragraph 3. Insert existing paragraph 4 in entirety as new paragraph 3.

Amendment 17 Delete paragraph 4. Insert the following:

- "
4. Preliminary Data Handling and Analysis.
 1. Four periods of data recording were recognised; i) Calendar week number 1-13, ii) week 14-26, iii) week 27-39, iv) week 40-52.
- Data sheets, and diskettes where applicable, should be prepared immediately after each recording period to cover the 13 weeks. Allowing for some preparation time, the country coordinator should forward these to the PLO at latest by the following times; Calendar week number 16, 29, 42, 03.
2. Wherever Protocol 1 has been changed, some additional extraction of data will be necessary. Master copies of new data will be necessary. Master copies of new data sheets will be prepared by the PLO and forwarded to all countries.

In certain cases, the meteorological data sets will need to be recompiled m start date in accordance with Protocol 1A.

3. Special summary data sheets will be prepared by the PLO for each data recording period. These will contain comparisons between trees and overall totals for various data sets over the whole period. These are to be completed and forwarded to the PLO with data sheets/diskettes. "

Amendment 18 Delete paragraph 5.1.a) and paragraph 5.1.b). Insert the following:

- "
5. Data presentation and storage
 1. Data Presentation

Histograms should be plotted using data sets from summary sheets 1A, 2A, 3A and 4A. Bars should be 10 mm wide for 2 week samples, and 5 mm wide for 1 week samples. These should be updated at the end of each recording period and used as the basis for reports from each site. "

3.4 PROTOCOL 1B OF THE 'COMPARATIVE EPIDEMIOLOGY EXPERIMENT'

At least two sites where Witches' broom is absent are due to be included in the 'Comparative Epidemiology Experiment'. It was necessary to develop a Protocol for such sites, derived from Protocol 1A. This is now called Protocol 1B.

3.4.1 Passport Data

Sites have been chosen, each representative of cocoa plantations from different regions without Witches' broom, and the following will be recorded.

a. Site

1. Geographic location
2. Latitude/longitude
3. Elevation above sea level (m)
4. Topography
5. Location of nearest establishment meteorological station
6. Photographs of site area

b. Plantation

A representative plantation will be selected, according to the interest of the participant, and the following recorded:

1. Area
2. Soil
3. Shade
4. Tree Age
5. Spacing
6. Plot location on map
7. Distance to other cocoa (plantations and/or wild cocoa)
8. History
9. Ground cover and litter
10. Cultural practices

c. Plot

10 representative sample trees will be chosen to form a plot within the plantation. These trees are to be selected in an area containing a minimum of 50 trees. They are assigned and marked with a number.

d. Trees

The following are recorded for each of the 10 marked trees:

1. Maximum height (m)
2. Trunk girth at 0.5m
3. Jorquette height/number
4. Canopy width/depth
5. Genetic material
6. Any peculiarities of the tree

3.4.2 Marking of trees and collection of data sets

a. Vegetative

Five units each of 10 healthy growing points are selected on each numbered tree representative of the canopy as a whole.

The following are recorded on these units every 2 weeks:

1. Number of flush points
2. Total number of flush leaves per unit

b. Cushions

Four 1m lengths on branches throughout the canopy and one 1m length in the middle of the trunk (50 cm if the trunk is short) are selected, marked and numbered on each tree.

The following are recorded within these lengths every 2 weeks:

1. Number of active cushions (active = when flower buds appear);
When cushion first shows activity it is marked with a pin.
2. Number of cushions with open flowers.

c. Fruit

The following are recorded every 2 weeks on the five 1m lengths designated for the cushion data sets and five additional 1m lengths in the branches of the tree:

1. Number of newly-set fruit > 1cm long. Each is marked with pin to indicate date observed.
2. Number of fruit wilted.
3. Number of healthy ripe fruit harvested.
4. Number of pods with necrosis. These are removed and counted. Phytophthora or any other causal agent should be noted if present.

For categories 2, 3 and 4 "the date of fruit set" is recorded. All other diseased and ripe pods on the trees in the plot are removed and counted every 2 weeks. The height of each pod to the nearest 50 cm is recorded on the ten sample trees.

. Meteorological

- i) Meteorological data are available from established stations near the designated plots.
- ii) The following equipment is placed outside the canopy of trees, in an open space, at each site:

Casella Thermohygrograph
Weighing bucket rain gauge
Belfort dew recorder
Maximum and minimum thermometer
Sling psychrometer"

2. It was suggested that :

- a. The Project Liaison Officer should determine the layout of the data sheets in consultation with a small number of interested participants at the Workshop. The layout of the sheets should reflect the need to transcribe data into to a form suitable for computing.
- b. Master copies of data sheets to be sent to sites for reproduction and preliminary use. Constructive criticism before final versions of forms are printed in bulk will be required. Printing in bulk could be undertaken centrally for all sites or within a country for its own sites.

3.4.4 Preliminary Data Handling and Analysis

- a. Four periods of data recording were recognised; i) Calendar week number 1-13, ii) week 14-26, iii) week 27-39, iv) week 40-52.

Data sheets, and diskettes where applicable, should be prepared immediately after each recording period to cover the 13 weeks. Allowing for some preparation time, the country coordinator should forward these to the PLO at latest by the following times; Calendar week number 16, 29, 42, 03.

- b. Special summary data sheets will be prepared by the PLO for each data recording period. These will contain comparisons between trees and overall totals for various data sets over the whole period. These are to be completed and forwarded to the PLO with data sheets/diskettes.

3.4.5 Data Presentation and Storage

1. Data Presentation

Histograms should be plotted using data sets from summary sheets 1A, 2A, 3A and 4A. Examples of these sheets are attached in Appendix C. Bars should be 10 mm wide for 2 week samples, and 5 mm wide for 1 week samples. These should be updated at the end of each recording period and used as the basis for reports from each site.

2. Data Storage

It was agreed that complete sets of data should be sent to and stored appropriately by IOCC and the Project Scientific Committee; additionally, data sets for each site within a country should be stored in that country.

3.5 ACTION TO BE TAKEN ON THE 'COMPARATIVE EPIDEMIOLOGY EXPERIMENT'

The following points were agreed and adopted:

1. PLO would investigate the computerisation of the IWBP relative humidity charts using a light pen and associated hardware;

2. All sites established in Year 1 were to be supplied with a sling psychrometer (manufactured by Bacharach Instruments, Pittsburgh, USA, for the calibration of the thermo-hygrographs unless those sites already had an operational psychrometer.
3. Publicity should be given by IWBP to the existence of Witches' Broom disease of cocoa on the island of St Vincent in the West Indies. This is a new record confirmed in a recent visit by Dr Sreenivasan to the island at the request of the authorities there. It is suggested that the pathogen may have been carried by a hurricane which affected the island about 5 years ago.
4. It was agreed that a site for the 'Comparative Epidemiology Experiment' should be established in Venezuela and that a complete set of equipment should be provided as soon as possible. The research site will be supervised by Dr Humberto and Dra Lilien Reyes and coordinated by Asdrobal Diaz MSc. The operator has not yet been appointed. It was agreed that the new site in Venezuela would be supplied with a Belfort Dew Recorder, Casella thermohygrograph, Rain gauge and sling psychrometer. The new sites in Brazil (Bahia) and Colombia (Santander) would each be supplied with a Belfort Dew Recorder only.
5. It was agreed that Protocol 1A would be circulated with all data sheets in their corrected form by the Executive Chairman in the series of IWBP Circular Letters.

4. INOCULUM AND/OR DISEASE GRADIENT EXPERIMENTS

4.1 INTRODUCTION

The value of disease gradient experiments in the IWBP project was accepted at the 1st Workshop and an objective was adopted to elaborate plans for disease gradient, sanitation ratio and other experiments. The following guidelines were initially prepared by Bob Schmidt, assisted by Gustavo Frias and, after discussion and more amendments, were agreed by all participants.

4.2 GUIDELINES FOR INOCULUM AND/OR DISEASE GRADIENT EXPERIMENTS

The following guidelines are intended to aid those wanting to establish inoculum and/or disease gradient experiments. These studies attempt to quantify the numbers of spores (spore gradients) and/or the amount of disease (disease gradients) as a function of distance from a known source of inoculum. The results of these studies can provide useful and necessary information for disease management, e.g. for phytosanitation. Because in nature these gradients depend on many factors such as the shape, strength and location of the inoculum source, climatic factors and topographic features, there is no one disease or inoculum gradient. Therefore many experiments are necessary. The following categories are important and will be discussed:

1. Inoculum source
2. Trap plants
3. Spore traps
4. Experimental Design
5. Data summary and Analysis

4.2.1 Inoculum source

The inoculum source should be isolated and of known size (shape) and strength.

- Use brooms which have the potential to produce basidiocarps
- Hang 3000 brooms on wires at two heights in a frame (approx 30m long and 2.5m high), shaded. Different sources are possible e.g. brooms on ground or a nearby infected plantation.
- In the absence of rain, brooms can be wetted artificially to stimulate basidiocarp production.
- The number of basidiocarps should be quantified during trap plant exposure (the average number of basidiocarps on 10% of the brooms should be counted each week during trap plant exposure).

4.2.2 Trap Plants

Trap plants should be seedlings with one or more susceptible infection courts free of Witches' Broom.

- Plants should be grown in individual containers in a place where they cannot be infected.
- Uniformly flushed plants should be exposed in field. The tops may be pruned to stimulate uniform flushing.
- Plants should be exposed for one flushing period (10 days).
- Plants should be watered at night from above and protected from sunlight during exposure. A roof can be placed over seedlings during the day and removed during the night when spore deposition occurs.
- Plants should be removed from the field following exposure and placed in a place where they cannot be infected with WB.
- Plants should be watered from below, without wetting infection courts. Approx. two months of post-exposure incubation are necessary to observe all infections which occur during exposure.

4.2.3 Spore Traps

When possible spore traps should be placed at plant infection court (shoot) height alongside plants during exposure.

- Roto-rod spore traps are appropriate (- these are volumetric and spores/m² of air can be calculated).
- Three to five traps are recommended.
- Time of exposure should be relatively short to avoid trapping too many spores. Preferably at maximum spore release i.e. 12.00 - 02.00 for 30-60 minutes.
- Identification of C. perniciosus spores must be verified by size and shape from trials and experience.

4.2.4 Design of Experiment

Trap plants are placed in replicated lines at various distances from the inoculum source (the spore traps are similarly placed).

- Two or three lines (replications) are required.
- Distances from broom inoculum source are commonly 1, 10, 20, 40, 80, 160, 320 and 640m (minimum 320m).

- Other distances may be useful, e.g. closer spacing at further distances might help to identify background contamination.
- At least 50 infection courts should be placed at each location on each line.
- Experiments may be repeated with new plants in order to obtain replication in time.
- The experimental area should be free of other sources of C.perniciosa inoculum, except for "normal" background levels.

4.2.5 Data Summary and Analysis

Data are summarised and graphed as percentage of plants (or infection courts if multiple infection courts) infected at various distances from the inoculum source. Often a linear transformation, e.g. log-log transformation, is useful to interpret the data. Specifically, the maximum distance of spread is the point at which the log-log line crosses the x axis or the level of background inoculum (point where log-log line changes slope).

5. EXPERIMENTATION ON CHEMICAL EVALUATION

5.1 INTRODUCTION

The possible role of chemicals in controlling Witches' Broom was examined in detail at the 2nd IWBP Workshop at Wageningen. It was agreed that certain aspects of the disease cycle e.g. basidiocarp production on dry brooms and infection of pods, might be particularly amenable to chemical control. So, to implement the agreed objective of the 1st IWBP Workshop "to elaborate plans for the examination of biocides" the following guidelines were prepared.

5.2. GUIDELINES FOR CHEMICAL EVALUATION EXPERIMENTS

The following are considered to be minimum requirements.

5.2.1 EXPERIMENTS UNDER CONTROLLED CONDITIONS

a. Shoots

Plant Material

Cut back seedlings of one genotype (c. 6 weeks old) to induce uniform flush points. Twenty-five plants should be used for each chemical tested.

Application

Soil drench (systemic activity) foliar (systemic); direct to flush point (systemic and protectant). One maximum dose or three dosages (high, medium, low). Applied in water in first tests, then possibly with surfactant/oil, either 18-24 hours before or 48-72 hours after inoculation.

Inoculation

Measured number of basidiospores in 0.25% water agar applied to each plant or measured amounts of known basidiospore concentration sprayed onto plants. Inoculum preferably derived

from basidiocarps obtained locally from several clones, though for convenience basidiocarps could be induced in cabinets or broom houses.

Evaluation

Formation of broom or not, within standard period compared with inoculated, untreated seedlings. If in the first test, less than 50% infection is obtained using a chemical then repeat test once or twice (maximum). Brooms formed in tests could be allowed to dry, hung in conditions suitable for induction of fruiting and productivity of basidiocarps assessed.

b. Brooms (antisporeulants)

Plant Material

- i) Dry, detached brooms with similar number of nodes, hung in cabinets or broom houses under conditions conducive to fruiting.
- ii) Dry, detached brooms suspended in tree canopy in the field.
- iii) Green brooms attached to tree.
- iv) Cushions brooms at relevant height on tree. Minimum sample of 20 brooms.

Treatment

Spray each broom once only with similar quantity of chemical. Apply to dry, not wet, brooms.

Evaluation

Count open, turgid basidiocarps weekly and note presence/absence of primordia. In cabinets, record in relation to unsprayed brooms over 6 months. In field, record productivity from dry brooms during one wet season; let green brooms dry and record productivity during next wet season. Count basidiocarps in the field once a week in a morning after rain. Remove open basidiocarps when counted.

Additional Investigations

Isolations from a small piece of broom onto a suitable agar medium to examine effect of chemical on internal mycelium. Tests of the viability of infectivity of basidiospores from basidiocarps induced on sprayed brooms.

c. Pods

Plant Material

Pollinations should be made during normal periods of natural pod set. Hand pollinate susceptible mother material with one pollen type. (Place tubes on flower buds night before pollination). Cover at pollination for 2 weeks, then if pod set, replace tube with plastic bag (cut at one bottom corner to allow rain water to drain). Plastic bags are only finally removed at 12 weeks. A minimum of 20 pods should be used for each treatment.

Inoculation

Inoculate pods at 6 weeks with basidiocarps on agar block, covered with wet cotton wool. Remove cotton wool after 24 hours. If all treatments cannot be inoculated at one time, then have a corresponding inoculated, untreated control for each inoculation date.

Application

Spray pod with chemical to run-off using a hand-held atomizer, either 18-24 hours before or 48-72 hours after inoculation. Label pods.

Evaluation

Record pods as infected or not infected. Number of healthy seeds can be counted or pods kept to assess basidiocarp productivity.

5.2.2 FIELD TESTS

Experimental Layout

Plots should be the following size: 7 x 7 (49 trees); one border row plus 5 x 5 'experimental' area. This is one replicate. There should be a minimum of 3 replicates of each of 5 treatments: a control (where either there is no treatment or water application with no chemical); a standard (1% Bordeaux mixture* applied at 55 L/ha); 3 test treatments. Therefore each experiment has 15 a minimum of plots (i.e. 735 trees).

Plots should conform to existing cultural practices over the whole site, which should be chosen as one with a good potential production. Before experiments begin, either all pods should be removed or those present marked.

(* mix only with fresh lime, a maximum of 5h before use).

Application

Chemicals should be applied to the central 25 trees in each plot using a motorized knapsack sprayer. Different makes of these are available in the various countries, and all specifications should be given. Number of applications, additives and volume of carrier will be determined by developing research. Minimum of 15 days between applications. Trials are to be conducted for a minimum of 2 years.

Evaluation

- 1) Pods. Assess on the whole tree, for each of the 25 trees in each plot. If all pods are removed or marked at the beginning of the trial, assessments can start immediately; otherwise start assessments 12 weeks after first spray application. Record number of healthy and diseased pods harvested every 2 weeks. Place diseased pods into standard categories, e.g. those with witches' broom, Monilia etc. all fermentable seed should be weighed.

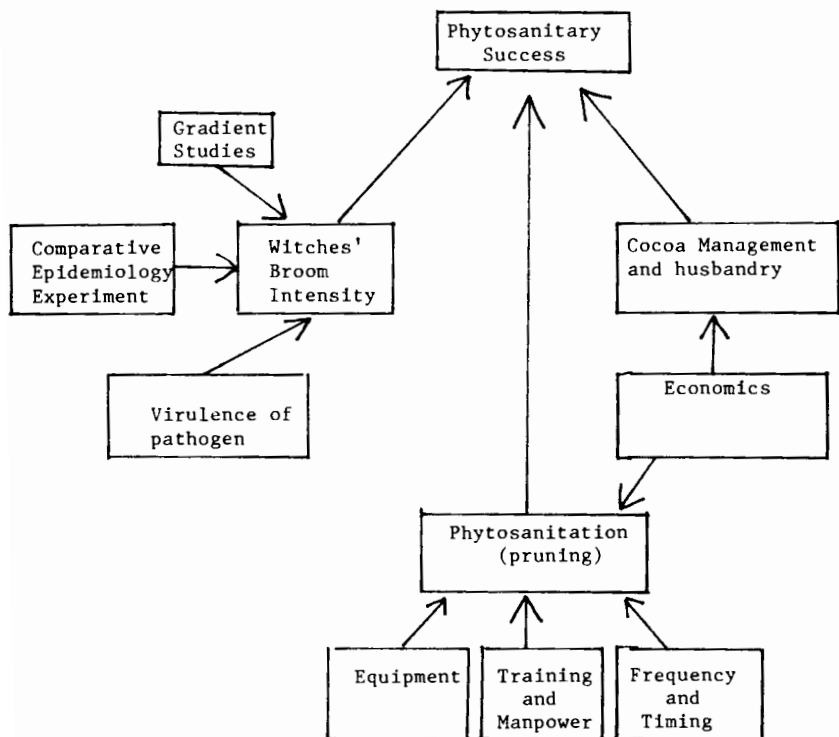
- ii) New Vegetative Brooms and Infected Cushions. Assess for whole tree, for each of the central 9 trees in each plot, every month. For each, use a scale: 1 = nil; 2 = 1-10; 3 = 11-50; 4 = 51-100; 5 = 101 - vegetative brooms or infected cushions per tree. (Extra classes may be added to extend scale). Start assessments one month after first spray application. If low scale values are obtained with chemicals, brooms or infected cushions can be counted.
- iii) Meteorology. Cumulative rainfall on daily basis; daily maximum and minimum temperature obtained from nearest standard meteorological station.

CAUTION

Protective Clothing. A minimum of hat, plastic eyeglass and plastic apron should be worn by spray operators.

6. PHYTOSANITATION EXPERIMENTS

The relationships between phytosanitation, the management and husbandry of the cocoa plantation and information about Witches' Broom from the IWBP project were first examined in detail. These relationships maybe described as follows:



Experiments based mainly on pruning could be established at different sites which data from the Comparative Epidemiology Project indicated were at different levels along the 'epidemic curve'. The importance of such experiments were stressed. They could provide invaluable information for disease management strategies. Although the disease status of the sites was indicated roughly now (e.g. Brazil - Altimira, low; Ecuador - Station, high, Trinidad - medium), it was generally agreed that it would be preferable to await more epidemiological data before attempting to prepare guidelines or a protocol for phytosanitary experiments. However, it would be particularly important to discuss phytosanitary experiments at the next IWBP Workshop when such data should be available.

7. ACKNOWLEDGEMENTS

Our thanks go to all participants in the 3rd IWBP Workshop for their interest and enthusiasm in particular to the various Moderators of the sessions and to Dr B E J Wheeler who again acted as rapporteur. In addition our sincere thanks to Dr Pablo Buritica and others of Instituto Colombiano Agropecuario (ICA) for the arrangements and for inviting us to hold the Workshop in Colombia. Particularly we would extend our sincere gratitude to the Directors of Chocolateria Luker for allowing us to hold the 3rd IWBP Workshop at Granja Luker and for so generously providing our lodging free of charge.

8. DATE FOR THE 4TH IWBP WORKSHOP

It is proposed to hold the 4th IWBP Workshop from 14th - 19th September 1987 or thereabouts in Belem, Brazil. Invitations will be issued nearer the time for appropriate participants. CEPLAC have kindly offered to act as hosts (with IOCC) for this meeting. Details of the Workshop arrangements will be confirmed closer to the time.

9. DATE OF THE MEETING OF THE PROJECT SCIENTIFIC COMMITTEE

It is presently proposed that the Project Scientific Committee should meet between 17-23rd May 1986 in the Dominican Republic during the course of the 10th International Cocoa Research Conference. Members of the Project Scientific Committee are invited to participate. The transportation and other costs of attending the 10th ICRC will not be funded by IWBP. Details will be confirmed closer to the time.

APPENDIX A: NAMES, ADDRESSES AND TELEPHONE NUMBERS OF PARTICIPANTS
IN 3RD IWPB WORKSHOP

[N.B. International dialling codes included in telephone numbers wherever possible]

Alberto Agudelo M., Depto Tecnico de Cacao, Chocolateria Luker, CRA
 23 No 20-40, Manizales, Colombia

Tel: : (57) 969.41455 (office); (57) 969.75032 (Granja)
 Telex: : 83586 LUKER CO

Teklu Andebrhan, CEPLAC/DEPEA, Caixa Postale 1801, Belem, PA, Brazil

Tel: : (55) 91.235.1800
 Telex : 91.1550. CPLC BR

Jennifer Andall, Cocoa Rehabilitation Project, Mount Horne, St
 Andrews, Grenada

Tel: : 1.809.444.7216 or 7757
 Telex: : None

Jaime Aragundi, INIAP, EET-Pichilingue, Apartado 24, Quevedo, Ecuador

Tel: : (593) 4.750.966
 Telex : (none at Pichilingue)
 Telex : INIAP Head Office in Quito 2532 (INIAP ED)

Fabio Aranzazu Hernandez, ICA, Apartado Aereo 876, Manizales,
 Colombia

Tel: : (57) 969.60552
 Telex: : 83588 ICAIX CO

Cleber Bastos, CEPLAC/DEPEA, Caixa Postal 1801, 66.000, Belem, PA,
 Brazil

Tel: : (55) 91.235.1800
 Telex: : 91.1550 CPLC BR

Pablo Buritica, ICA, Apartado Aereo 151123, Eldorado, Bogota,
 Colombia

Tel: : (57) 2860.425
 Telex: : 44309 ICATE CO

Bernardo Saenz Cardona, Apartado Aereo 3938-30244, Fedecafe, Bogota,
 D.E. Colombia

Tel: : (57) 2835.100 Ext 210
 Telex: : 41281

Sergio Cadavid V., Compania Nacional de Chocolates, Apartado Aereo
 717, Medellin, Colombia

Tel: :
 Telex: :

Carlos Coral S., ICA La Libertad, Villavicencio, Meta, Colombia

Tel: : (57) 9.867162 (Regional Office)
 Telex: : 46648 ICAVO CO (Regional Office)

Gustavo A Frias, Plant Pathology Dept, University of Florida,
 Gainesville, Florida, 32611, USA

Tel: : 1.904.392.3639
 Telex: : None

Robert H Fulton, 15501 SW 79th Avenue, Miami, Florida 33157, USA

Tel: : (305) 238.6428
Telex: : None

Tony Lass, Cadbury Schweppes plc, Bournville, Birmingham B30 2LU, UK

Tel: : Work (44) 21.458.2000 Ext 4917
Tel: : Home (44) 92.684.3489
Telex: : 338656 CADBUY G

Alan Maddison, INIAP EET Pichilingue, Apartado 24, Quevedo, Ecuador

Tel: : (593) 4.750.966
Telex: : (None at Pichilingue)
Telex: : INIAP Head Office in Quito 2532 (INIAP ED)

Francisco Ilton Morais, CEPLAC/DEPEA, Caixa Postal 1801, 66.000,
Belem, PA, Brazil

Tel: : (55) 91.235.1800
Telex: : 91.1550 CPLC BR

Humberto Reyes E, FONAIAP-CENIAP, Apartado Postal 4653, Zona Postal
2101, Maracay, Estado Aragua, Venezuela

Tel: :
Telex: :

Lilian Capriles de Reyes, FONAIAP-CENIAP, Apartado Postal 4653 - Zona
Postal 2101, Maracay, Estado Aragua, Venezuela

Tel: :
Telex: :

Francesco Ocampo Rojas, ICA CNI Palmira, Apartado Aereo 233,
Palmira, Colombia

Tel: :
Telex: :

Hank Purdy, Plant Pathology Dept, IFAS, University of Florida,
Gainesville, Florida 32611, USA

Tel: : (1) 904.392.7235
Telex: : None

Jose Guillermo Rondon, CRI Tulenapa - ICA, Chigorodo, Antioquia,
Colombia

Tel: : None
Telex: : None

Stephen A Rudgard, CIBC, Silwood Park, Ascot, Berkshire, SL5 7PY, UK

Tel: : (44) 990.28108
Telex: : 265871 MONREF G quoting 84: CAU015

Bob Schmidt, Dept of Forestry, School of Forest Resources, IFAS,
University of Florida, 214 Newins-Zeigler Hall, Gainesville, Florida
22611, USA.

Tel: : Work : (1) 904.392.4826
Home : (1) 904.378.8615
Telex: : None

T N Sreenivasan, Cocoa Research Unit, University of the West Indies,
St Augustine, Trinidad, West Indies

Tel: : (1) 809.663.1359
Telex: : 24520 UWI WG

Hille Toxopeus, SVP, P O Box 117, 6700 AC Wageningen, The Netherlands

Tel: : (31) 8370.19112
Telex: : 45888 INTAS Marked 'For SVP please'

Bryan Wheeler, Imperial College, Silwood Pk, Ascot, Berks SL5 7PY, UK

Tel: : (44) 0990.23911
Telex: : 261503 IMPCOL G

APPENDIX B

PROGRAMME OF 3RD IWBP WORKSHOP
GRANJA LUKER, CHOCOLATERIA LUKER,
SANTAGUEDA, MANIZALES, CLADAS, COLOMBIA

Sunday 6th July 1986

Participants arrive at Granja Luker.

Monday 7th July 1986

OPENING SESSION

- 08.00 Welcome by representative of ICA (Pablo Buritica)
- 08.10 Introduction of Participants
- 8.20 Welcome by Director for ICA Region 9 (Rafael Estrada)
- 08.30 Welcome by representative of Chocolateria Luker
(Alberto Agudelo Munoz)
- 08.40 Welcome by representative of IOCC (Tony Lass)
- 08.50 Welcome by Chairman of Project Scientific Committee
(Hank Purdy)
- 09.00 Adoption of Agenda for 3rd IWBP Workshop
Adoption of Objectives of 3rd IWBP Workshop
Appointment of Rapporteurs
- 09.10 Discussion on Objectives of IWBP
- 09.30 Progress on IWBP to date (Stephen Rudgard)

SESSION I

- 09.45 "COMPARATIVE EPIDEMIOLOGY EXPERIMENT"
(Moderators: Alan Maddison, Stephen Rudgard,
Bob Schmidt)
 - Review of epidemiological data sets by country to
June 1986
 - Discussion and reaffirmation of objectives of
comparative epidemiological experiments
 - Modifications and alterations to Protocol I
 - Discussion on data recording at sites where Witches'
Broom is absent
- 18.00 Session adjourned
- 20.00 "CHEMICAL CONTROL" (Moderator: Hank Purdy)
Background to chemical control of plant diseases
(Bob Fulton)
- 22.00 Session adjourned

Tuesday 8th July 1986

SESSION I (continued)

- 08.00 "COMPARATIVE EPIDEMIOLOGY EXPERIMENT" (concluded)
- 12.15 Session adjourned

SESSION III

- 13.30 DISEASE GRADIENT EXPERIMENTS
Experiment in Brazil : Stephen Rudgard
Experiment in Ecuador : Gustavo Frias
- 15.30 Session adjourned

SESSION IV

- 15.45 PHYTOSANITATION AND ISOLATED PLOT EXPERIMENTS
CEPLAC Demonstration plot in Ouro Preto: Stephen Rudgard
Pruning Exp. in Benevides, Brazil: Teklu Andebrhan
- 18.00 Session adjourned
- 20.00 - 22.00 Working groups

Wednesday 9th July 1986

SESSION II - Field Trip

- 06.00 Visit 'La Finca Argentina'
Visit to IWBEP Experimental Site
Visit to Granja Luker
- 14.00 SESSION IV (continued) (Moderator: Hank Purdy)
Chemical control experiments in Brazil: Cleber Bastos
" " " Venezuela: Lilien Reyes
" " " Colombia: Guillermo Rondon
Ecuador: Alan Maddison
- 18.00 Session adjourned
- Dinner hosted by Compania Nacional de Chocolates

Thursday 10th July 1986

- 07.30 Meeting of Project Scientific Committee
- 09.00 Adoption of Protocol IA on 'Comparative Epidemiology Experiment'

SESSION IV (continued)

- 10.15 Preparation of Guidelines for Chemical Evaluation
(Moderator: Stephen Rudgard)
- 3.45 Preparation of Guidelines for Chemical Evaluation
(continued)

- 15.30 Inoculation Techniques using Basidiocarp
Suspensions: Hank Purdy
- 16.30 Infection Process and Methods for Resistance
Screening: Gustavo Frias
- 18.15 Session adjourned
- 19.30 Discussion on Interpretation of Dew Recorder Charts
- 20.00 Discussion on Disease Management Strategies

Friday 11th July 1986

- 07.30 Meeting of Project Scientific Committee (concluded)
- 09.00 Adoption of Guidelines for Disease Gradient
Experiments
- 10.30 Adoption of Protocol IA (concluded)
- 14.00 Pruning trials in Colombia: Fabio Aranzazu
- 15.15 Chemical control trial in Ecuador: Jaime Aragundi
- 16.00 Witches' Broom in Grenada: Jennifer Andell
- 16.15 Adoption of 'Guidelines on Chemical Evaluation'
- 16.30 Closure of the Workshop
- 19.00 Social Event hosted by IOCC
Vote of thanks to Chocoletaria Luker: Ilton Morais

Saturday 12th July 1986

Meetings and Field Visits with cocoa farmers and staff of Federacion Nacional de Cafeteros de Colombia.

Departure of Participants.