

**FINAL REPORT OF THE INTERNATIONAL WORKSHOP  
MONILIASIS, REGIONAL BREEDING AND RESISTANCE**

**CATIE, Turrialba, Costa Rica  
21-25 February 2005**

**EVENTS**

**1. SHORT AND MEDIUM-TERM STRATEGIES TO AVOID A FROSTY POD  
ROT PANDEMIC IN CENTRAL & SOUTH AMERICA**

**Supported by Masterfoods, USDA and CATIE  
Technical coordinator: Dr. Prakash Hebbar**

**2. THE AMERICAN CACAO BREEDING PROGRAM (ABP) PLANNING AND  
DISCUSSION MEETING**

**Supported by Masterfoods, USDA, WCF, CFC/ICCO/IPGRI Project, CATIE and  
National and Regional Cacao Research Institutions in the Americas  
Technical coordinator: Dr. Juan Carlos Motamayor**

**3: RESISTANCE TESTING METHODS MEETING**

**Supported by CATIE, CFC/IICO/IPGRI, Masterfoods, and USDA  
Technical coordinator: Dr. Bertus Eskes**

**Local Organizer:  
Dr. Wilber Phillips, CATIE**

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**1. REPORT OF THE SHORT AND MEDIUM-TERM STRATEGIES TO AVOID  
FROSTY POD ROT PANDEMIC IN CENTRAL & SOUTH AMERICA**

**CATIE, 21-22 FEBRUARY 2005**

*With support from Masterfoods, USDA, and CATIE*

**1.1. OBJECTIVE:**

To formulate an action plan to contain the spread of Frosty Pod Rot (FPR) in Central and South America.

**1.2. STRATEGY OF THE MEETING:**

A group of experts in plant pathology, breeding, epidemiology, IPM, extension, and farmer training met to discuss the feasibility of short-term interventions to prevent FPR from becoming a pandemic in Central and South America and the Caribbean region. The findings summarized below will be recommended for Governments to act upon from tropical America currently free (Brazil, Bolivia, Dominican Republic, Trinidad and Tobago, Mexico, etc) and those recently affected by the Frosty Pod disease.

**1.3. RECOMMENDED NEXT STEPS:**

- a) A letter to be sent to the Ministers of Agriculture in tropical America with signatures of attendees of this meeting, representing countries that have been recently affected, those that are threatened, and those that already have FPR disease.
  - b) Synthesize the important next-steps through an action plan including general recommendations to Ministries of Agriculture (see below).
  - c) Outreach/Public awareness of stakeholders, through a series of events in the next few months,
    - Ministry of Agriculture, through CATIE Board-Council superior
    - ACCESSO meeting, Lima, Peru, USAID, US State Dept., USDA, WCF, OAS-CICAD
    - ICCO meeting, Ecuador.
    - Malaysian Cocoa Board meeting, July 2005.
    - GTZ regional program.
    - COPAL meeting at CATIE, Costa Rica, February 2006
- PARTICIPANTS WITH THEIR RESPECTIVE GOVERNMENTS.
- All the stakeholders, such as the Industry, donors (USAID, US State, GTZ), NGO's will be informed of the recommendations emanating from the meeting.

## 1.4 ACTION PLAN FOR CENTRAL AND SOUTH AMERICA AGAINST FROSTY POD ROT

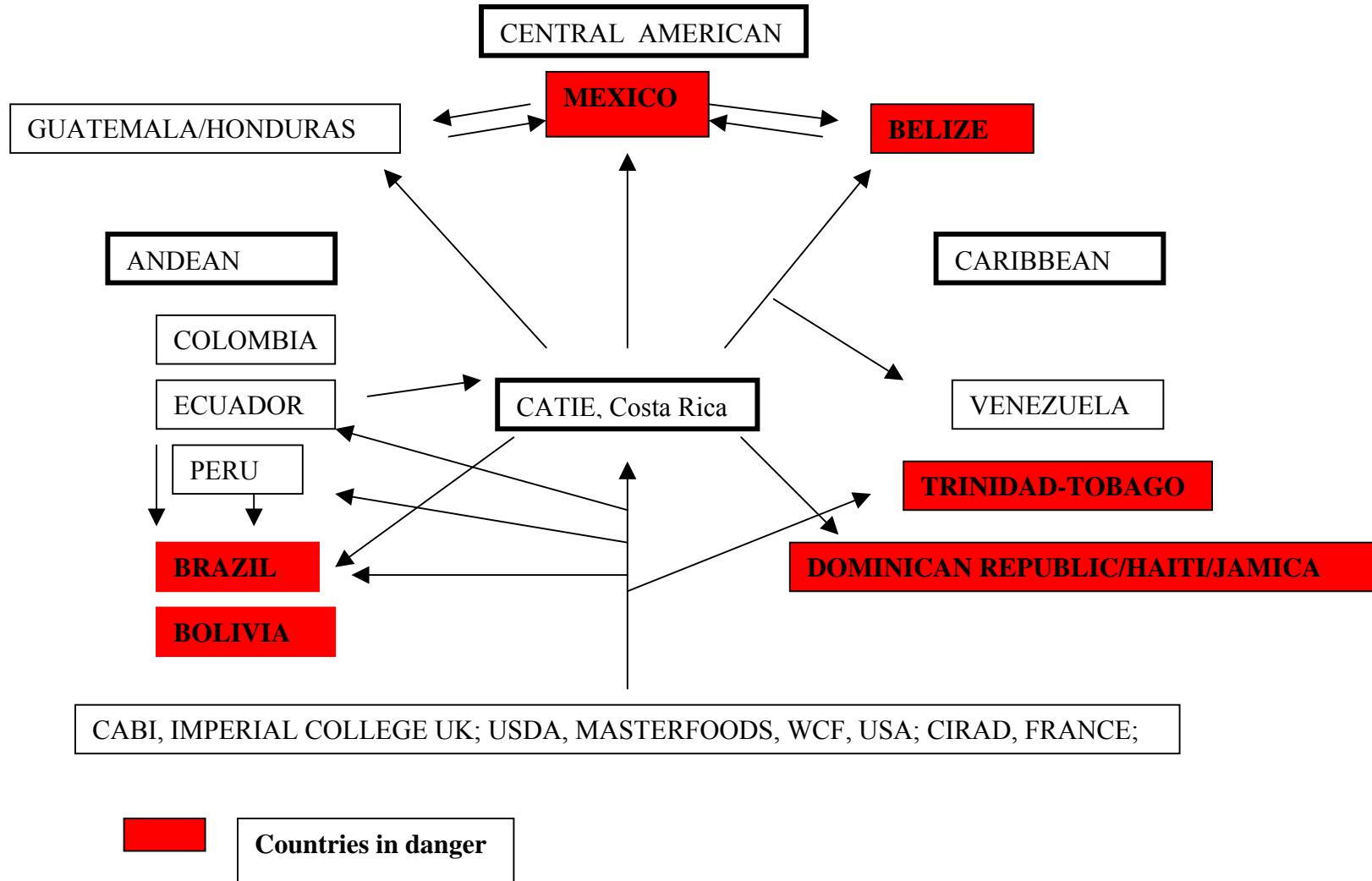
### I.4.1 Belize/Mexico/Guatemala.

Country	Eradication Quarantine	Public Awareness	Monitoring	Farmer Training	Epidemiology	ICM/Biocontrol	Breeding/Genetics	Funding
<b>Belize</b>	1-Continue eradication, on sites surveyed by G&B 2-Destroy pods, destroy trees later? 3-Compensate farmers.	1-Contact OSA: Central American Quarantine. 2-Ministry of Agriculture. 3-Coordinate activities with the Mexican authorities	1-Continue monitoring.	1-Train technicians CATIE. 2-Involve farmers. 3-need urgently literature on FPR to be distributed. 3-Network with other countries that have the disease.	1-send pathogens to USDA for fingerprinting. 2-Host range, disease vectors.	1-Release known biocontrol agents effective against FPR. 2-Network and receive info from Ecuador/Peru/CATIE on ICM.	1-Introduce new resistant materials from CATIE, Ecuador, through Reading Quarantine	1-Contact stakeholders DIFID, BCCCA, OAS, Green and Black, Belize Govt.
<b>Mexico Guatemala</b>	1-Strict Quarantine Guatemala, Belize border. 2-Stop importation of pods and infected beans to delay disease entry	1-Inform and involve cacao industry. 2-Contact OSA, a regional+Brazil and Bolivia project. 3-Pressure on Govts.	1-monitor farms. 2-Info on extent of FPR in Guatemala needed.	1-Train farmers to recognize the disease. 2-Reduce tree height now as a precaution. 3-ICT offers assistance.	1-Info on host range, disease vectors. 2-Crop loss estimates?	1-Receive info from Ecuador/Peru/CATIE on ICM. 2-Rehab farms in Chiapis and Tabasco, first step to farmer organization	1-Plant or transfer materials now with known resistance. 2-Transfer of tissue cultured plants would be quicker.	1-\$1.0 million proposal sent to the govts. Of Chiapis and Tabasco. 2-Coordinate with Guatemala and Belize, regional initiative.

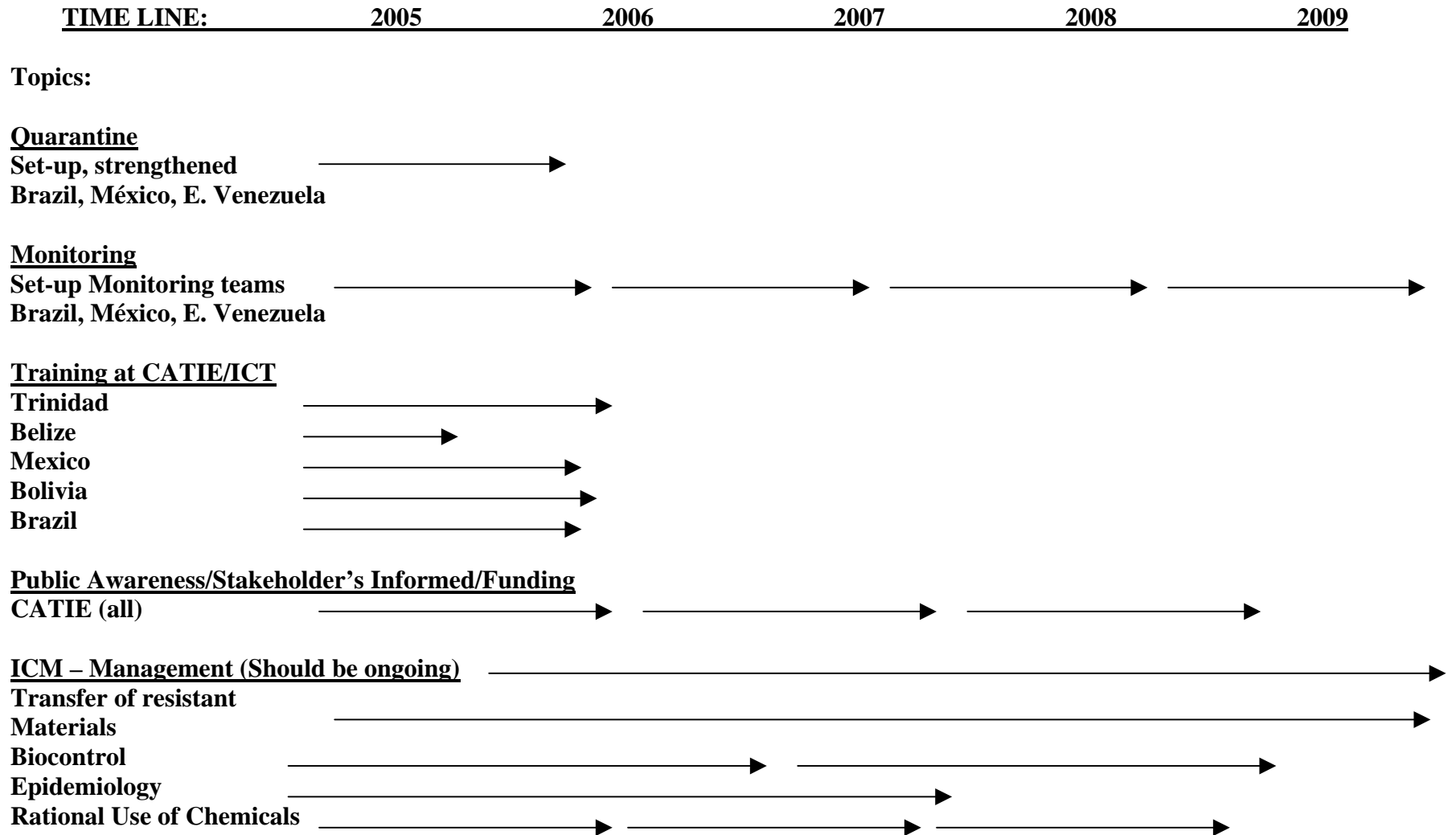
### 1.4.2. Caribbean and South America.

Country	Quarantine	Public Awareness	Monitoring	Technicians/Farmer Training	Epidemiology	ICM/Biocontrol	Breeding/Genetics	Funding
Brazil Peru Bolivia	<p>1-<u>Strengthen Quarantine at 5 potential entry points.</u></p> <p>2-Involve SENASA, Peru Quarantine.</p> <p>3-be aware of human involvement of disease movement.</p>	<p>1-<u>Workshop recommendations to be sent to Min. of Ag. of Brazil and Peru as soon as possible.</u></p> <p>2-CEPEC Bahia and Belem to contact CEPEC Director.</p> <p>3-Initiate discussion PR on FPR in Brazil for stakeholders.</p>	<p>1-<u>Initiate CEPEC, UNAS, ICT, Almirante, CABI survey now!!</u></p> <p>2-Set-up spore traps as soon as it is available.</p> <p>3-ICT offers lab facility.</p> <p>4-official letters need.</p>	<p>1-Train technicians at <u>CATIE/ICT.</u></p> <p>2-FPR manual in Portuguese has been distributed.</p> <p>3-Update training manual. UNAS updated version.</p> <p>Collate information from all manuals produced in other countries.</p> <p>4-WCF involvement virtual library.</p> <p>5-Cargill to supply info to farmers-supply chain involvement.</p>	<p>1-send <u>pathogens collected to USDA for fingerprinting</u></p>	<p>1-Network and receive info from Ecuador/Peru/ CATIE on ICM.</p>	<p>1-<u>Preventive breeding has been initiated in collaboration with Peru.</u></p>	<p>1-Contact stakeholders, cacao industry, Brazilian Govt., farmer groups.</p> <p>2-Pocitropicos proposal has been sent by INIAP for a regional project.</p>
Venezuela Trinidad Tobago Dominican Republic, Haiti, Jamaica	<p>1-<u>Strengthen Quarantine in E. Venezuela.</u></p> <p>2-<u>Stop movement of infected pods, &amp; plants to E. Venezuela</u></p>	<p>1- Involve cacao industry.</p> <p>2- Airport/Seaport quarantine, information on FPR</p> <p>3-<u>Caribbean regional coordination</u></p>	<p>1-<u>monitor farms in W. Venezuela</u></p>	<p>1-Training manual needed in English.</p> <p>2-<u>CATIE training of technicians</u></p> <p>3-<u>Reduce tree height now as a precaution</u></p>	<p>1-Send pathogens collected to USDA.</p>	<p>1-Network and receive info from Ecuador/Peru/ CATIE on ICM.</p>	<p>1-<u>Plant or transfer material with durable resistance.</u></p> <p>2-CRU can facilitate</p> <p>2- tissue cultured plants-Almirante offers assistance.</p> <p>3-Fingerprinting at CATIE</p>	<p>1-No identified funding source.</p> <p>2-CFC project on regional breeding.</p>

### 1.5 ORGANIZATION OF SHORT-TERM STRATEGIES FOR FROSTY POD ROT MANAGEMENT & COUNTRIES INVOLVED



## 1.6 ACTIVITIES CHRONOGRAM



## **2. REPORT ON THE AMERICAN CACAO BREEDING PROGRAM (ABP) PLANNING AND DISCUSSION MEETING**

**CATIE, 23-24 FEBRUARY 2005**

*With support from Masterfoods, USDA, WCF, CFC/ICCO/IPGRI Project, CATIE and  
National and Regional Cacao Research Institutions in the Americas*

**Report prepared by**

David Iwaro (CRU)

### **2.1. OBJECTIVES**

- Discuss the organization of a Regional Cacao Breeding Initiative in America, based on national and regional priorities.
- Identify practical linkages between different breeding initiatives in progress in the region, and coordinate joint efforts to get more efficient and successful programs.

### **2.2 INTRODUCTION TO THE MEETING**

At the beginning of the meeting, Roger Dehnel made a presentation emphasizing the following issues:

- The need to share technologies/solutions and germplasm
- Provide support to finalize ongoing activities and establish advanced trials
- Add to potential to achieve transfer to farmers
- Avoid duplication of activities

Juan Carlos Motamayor mentioned the following objectives and expectations for the meeting:

### **2.3 PRESENTATIONS GIVEN**

- i) Nine presentations were made on National Cacao Breeding Programs in 8 countries (Venezuela, Peru, Trinidad, Costa Rica, Honduras, Mexico, Brazil and Ecuador). Progress achieved and difficulties encountered in the various programs were highlighted in the presentations.
- ii) Two presentations were made on the USDA/Masterfoods Cacao Breeding Programs in Ecuador and Costa Rica
- iii) Presentations were also made on CFC Cacao Breeding Programs

- USDA+CFC breeding activities in Brazil
- CRU pre-breeding activities in CRU, Trinidad

On Thursday the following fundamental issues related to the operation of the ABP were discussed at the meeting. The results reached at the plenary and group discussions are reflected hereafter.

## **2.4. MISSION OF THE ABP**

***Vision:*** To establish a virtual program where members representing different institutions could lead different activities based on comparative/competitive advantages

***Mission:*** To facilitate on-going national programs taking advantage of the comparative/competitive advantages of the different participating institutions within the region

## **2.5 PROBLEMS THAT SHOULD RECEIVE ATTENTION FROM THE ABP**

### **2.5.1 Problems identified that could be solved through genetic improvement:**

- Low yields
- Losses due to Moniliasis, Witches' broom, Black Pod, Ceratocystis wilt
- Quality
- Size and shape of trees

### **2.5.2 Problems associated with the implementation of breeding programs**

- Difficulties associated with exchange of germplasm (laws + methods)
- Lack of training
- Lack of coordination
- Lack of standardized methods for selection
- Lack of improved planting material
- Small proportion of resistant material is available.

## **2.6 LIST OF ON-GOING ACTIVITIES WITH REGIONAL INTEREST (NOT NECESSARILY COORDINATED AT THIS TIME AT REGIONAL LEVEL)**

- Population improvement for Moniliasis in Costa Rica
- Population improvement for WB in Ecuador and CRU
- Population improvement for BP in CRU
- CFC-Biomol Program (CEPLAC, INIAP, ICT)

- CFC Regional variety trial
- CFC+USDA replicated QTL trial
- Molecular characterization of collections (USDA)

*It was agreed that coordination between these activities through ABP could improve their efficiency*

## **2.7 SUGGESTED AREAS FOR PRIORITY IN THE ABP**

- The collection of germplasm in Peru is important for the region, and an effort should be made to evaluate the collected germplasm
- The CRU quarantine facility in Barbados could be upgraded to facilitate the movement of germplasm within the region. It was noted that the Reading facility is currently full
- It was agreed that various collections within the regions should be evaluated for resistance to Moniliasis
- The search for resistance to Moniliasis in Colombia was proposed as one of the activities that should be carried out within the ABP
- The participation of the farmers in the search for resistance to Moniliasis was noted.

*Recognizing the present impact of Monilia on cacao production within the region and the future risk if not controlled, participants at the meetings unanimously agreed that the control and prevention strategies of this disease through breeding be given high priority on the agenda of the Latin American Breeding Program.*

*Participants agreed to support breeding activities related to the control and prevention strategies of Moniliasis as the first project to launch the regional collaboration.*

### **The objectives of this activity would be:**

- *To coordinate all activities on Moniliasis resistance in the region*
- *To support CATIE and NARS Moniliasis resistance programs (through coordination)*
- *To improve germplasm exchange situation*
- *To support Local trials within the region*
- *To support the utilization of the introduced material in national breeding programs (enhancement of current activities + preventive breeding)*
- *To support research to establish new methodologies for early screening of the disease (molecular markers, phloem sap, etc.)*

## 2.8 REPORTS FROM WORKING GROUPS

Four working groups were formed to prepare working procedures for the following activities related to the operation of the ABP

- Coordination
- Exchange of Germplasm
- Utilization of resistant germplasm
- Methodologies of screening for resistance.

### 2.8.1 Coordination aspects

A schematic on the decision making process is shown in Table 1.

### 2.8.2 Exchange of Germplasm

The members of this working group discussed the following aspects:

#### a. Quarantine

Where?

Location:

- CRU -Barbados (resources needed for upgrading of facility and maintenance)
- CENARGEN- Brazil (Functional)
- USDA-Miami (functional, and could be an alternative to Barbados)

Time for regional quarantine??

It was concluded that more research is required to determine the minimum required time to maintain the plants in the quarantine

#### b. Methods

How?

- Cuttings or Micro-grafting
- Tissue culture (approach Penn State University for the state of art and possibilities with tissue culture or somatic embryo genesis)
- Need to develop methods for early diagnosis of diseases

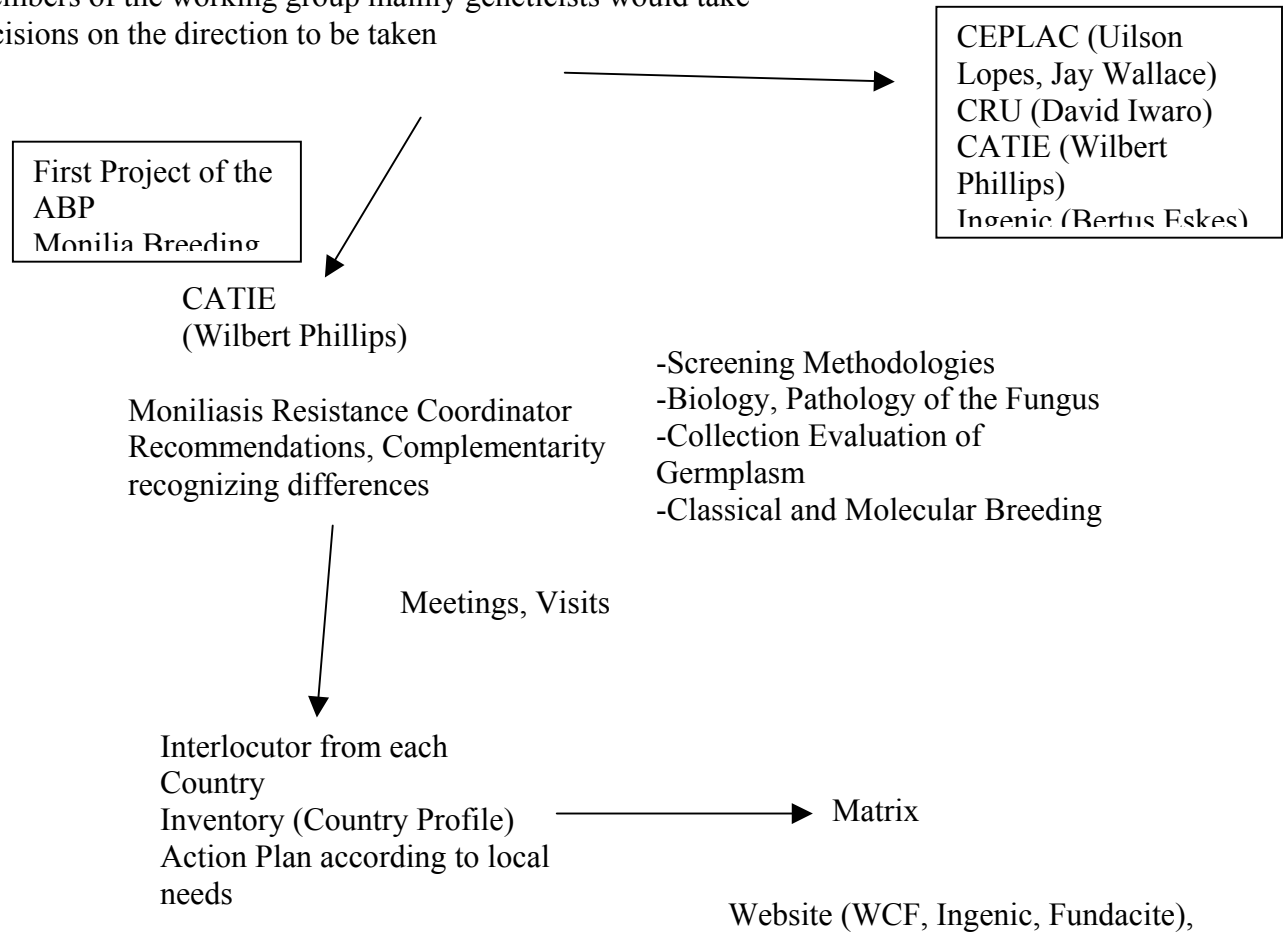
#### c. Material and Regulations (IPGRI regulations on transfer of cacao germplasm – IPGRI web site)

What?

- List of clones available in country members
- Clone names
- Country legal regulations: bilateral agreement between countries
- Materials available in Reading and other places
- Include clone characteristics (Agronomic etc)

**TABLE 1: WORKING GROUP/ DECISION MAKERS**

Members of the working group mainly geneticists would take decisions on the direction to be taken



### 2.8.3 Methods of screening for resistance to frosty pod disease

The group deliberated on the following aspects of screening for methodology:

- a) Existing methods applied on pods
  - Spray method
  - Patch method
- b) Problems encountered
  - Low spore germination
    - o Limited understanding of the life cycle of the pathogen, in particular, change from the parasitic to saprophytic phase
- c) Genetic diversity within pathogen population
  - 3 genetic groups of isolates have been identified within the region
    - o It was recommended that more isolates be collected, particularly from Colombia to provide a full range of pathogen variability for assessment of diversity.
    - o It was also recommended that a wide range of resistant (ICS 95, EET 233, EET 75, PA 169, UF 273 and UF 712) and susceptible genotypes be included in tests to pathogen aggressiveness.
- d) Potential screening methods
 

It was recommended that the potential of the following methods be assessed as possible early screening methods:

  - Spores germination on callus
  - Spores germination in pod extracts
  - Molecular marker (an F2 population in Miami could facilitate this study)
- e) The meeting recommended a ring test to assess possible interaction between genotypes and isolates.

### 2.8.4 Utilization of resistance

The following priorities were proposed:

1. Immediate introduction of resistant material in the country. This should be evaluated locally in the most suitable way (in collections or in clone trials). Consider not only budwood but also alternative methods: somatic embryos, seed, and pollen.
2. Select for trees that have a favorable canopy, facilitating cultural and chemical control measures. Such canopies should be open, relatively low, and easy to prune. This type of canopy is expected to reduce disease incidence in relation to tall and dense canopies. This is a type of preventive breeding.
3. Select for durable resistance to moniliasis. There is a need to confirm stability of resistance and study on hypersensitive.
4. Combine resistance to monilia with other important traits, such as resistance to witches' broom, yield and quality traits. This can only be done in countries where these

traits can be selected. The possibility of a centralized approach to multiple trait selection could be considered if the resistance is stable over different sites. This has to be evaluated.

5. Search for new sources of resistance to moniliasis, not only in collections but also in farmers' fields, using indigenous knowledge.

## **2.9 NEXT STEPS**

The working group identified in 8.1 will work in setting up the frame of the collaboration, which will be sent to the participating countries:

- Brazil (CEPLAC Rondonia and Bahia units)
- Trinidad and Tobago (CRU)
- Costa Rica (CATIE)
- Honduras (FHIA)
- Venezuela (INIA)
- Mexico (INIFAP)
- Dominican Republic
- Colombia? (Corpoica?)
- Peru (Universidad de La Selva, ICT)
- Ecuador (INIAP)

The coordination of the working group can rotate yearly; an individual or an institution could represent the coordination of the working group. Juan Carlos Motamayor has volunteered to coordinate the working group for this year.

Once the frame of the ABP is officially established and the first project of the regional collaboration, the objective of which is to coordinate the genetic improvement for frosty pod resistance be prepared in detail, research for funds will be carried out to implement the program. One of the possible sources of funding is the Dutch Buffer Stock Fund. Juan Carlos Motamayor and Edgardo Monteverde will be working on this proposal during the next weeks.

If resources are available a meeting together with the next disease resistance methodologies meeting next year will be carried out. Significant progress on the establishment of the network is expected for that time.

### 3. REPORT OF THE RESISTANCE TESTING METHOD MEETING

**CATIE, 24-25 FEBRUARY 2005**

*With support from CATIE, CFC, Masterfoods and USDA*

#### **3.1 SCOPE OF THE MEETING**

Resistance testing is a fundamental element in guiding and speeding up breeding programs, which have resistance as a main selection criterion. The objectives of the "Resistance Testing Method Meeting" are related to the Component 2.4.1 of the CFC/ICCO/IPGRI project, including activities supported by the CFC as well as by co-financing institutions (CIRAD, Masterfoods, USDA, WCF) and counterpart contributions. The meeting is technical and should benefit from being informal.

#### **3.2 OBJECTIVES**

- Compare reliability of results obtained for monilia, witches' broom and black pod resistance testing;
- Analyze proposals for more rapid or more efficient resistance testing of adult plants;
- Analyze proposals for early resistance testing methods;
- Finalize Working Procedures on resistance testing methods to be adopted in the new CFC/ICCO/IPGRI project in the Americas; and
- Agree activities to be carried out in component 2.4.1 over the next two years (2005 and 2006) of the CFC/ICCO/IPGRI project.

#### **3.3 STRATEGY OF THE MEETING**

Each of the institutions presented:

- Information on the correlation of results of "traditional" resistance testing methods with field level of infection,
- Ongoing research initiated on "alternative" resistance testing methods,
- Comments on draft Working Procedures prepared for WBD and monilia resistance testing (according to documents distributed to the participants), and
- Proposals for studies to be developed over the next 2 years.

The discussions were directed to obtain agreement on:

- Working procedures for the CFC/ICCO/IPGRI Project Activity 2.4.1, and

- Collaborative and other activities that will be carried out at each institution over the next two years.

### 3.4 OUTPUTS REACHED

#### 3.4.1 Conclusions on reliability of resistance testing methods

##### *Moniliasis*

The attached pod inoculation method has given reliable results in Costa Rica, Ecuador and Peru, when comparing field infection levels of the disease with resistance measured by the inoculation test (as internal severity). Incubation period and latency period seem correlated to internal severity. In Costa Rica, it was shown that this method can be highly efficient in selecting individual adult trees. The method is however labor-intensive and can only be applied to adult plants. There is therefore an urgent need to develop reliable early screening methods for resistance to moniliasis. Research in this direction has been initiated at CATIE.

A presentation was given on results obtained in the 1980's in *Ecuador*, using inoculation of pre-germinated seeds with a high concentration of spores. Distortion of growth was noticed in a large percentage of seedlings. Several seedlings also developed dark spots and necrosis on the outer layer of the hypocotyls or on the stem bark tissue. The fungus could be successfully isolated from such tissues, even in later stages. These results show the potential of developing an early screening test for resistance to moniliasis.

##### *Phytophthora*

Results were presented on attached and detached pod inoculation as well as on leaf disc inoculations.

In *Trinidad*, the detached pod test, using a 0 to 8 rating scale for severity of infection, proved to be significantly correlated ( $r = 0.67$ ) with field observations done on 40 clones over a three-year period. The correlation between the detached pod test and the leaf disc test appeared to be influenced by the scaling (slightly significant for the scores between 5 and 8 for the pod test, and non-significant for the entire 0-8 point scale).

In *Mexico*, a highly significant correlation was found between the field infection level of 10 clones and the scores in the leaf disc test ( $r = 0.86$ ).

In *Brazil*, a significant but relatively low correlation ( $r = 0.25$  to  $0.40$ ) was found between field levels of attack and results obtained with the leaf disc test. No significant correlation was found for 11 clones inoculated with three *Phytophthora* species in leaf discs, attached and detached pods. In general, resistance to the *P. citrophthora* species was not well correlated with resistance to the other two species tested (*P. capsici* and *P.*

*palmivora*). This may be a factor that affects the correlation between inoculation tests and field infection.

In *Costa Rica*, a significant correlation between field incidence and attached pod inoculations was found ( $r = 0.54$ ). Preliminary results with the leaf disc test did not show a significant correlation between this test and the attached pod test.

It can be concluded that significant correlations between field results and different type of inoculation tests can be obtained, indicating that the use of these tests can be efficient in selecting for more resistant genotypes. There was no need expressed for further developing new resistance methods for *Phytophthora*. The correlation between different inoculation tests was not always significant. It could therefore be that each test reveals slightly different components of the genetic resistance mechanisms to *Phytophthora*.

### **Vegetative resistance to witches' broom**

#### ***Traditional methods***

In *Brazil*, the % of infected seedlings inoculated with the belt-spray method was significantly correlated with the number of healthy plants in the field ( $r = 0.33$ ) and with the time that plants stayed healthy after field planting ( $r = 0.11$ ). There was no significant correlation with the number of brooms observed on the field plants. Many clones could be selected for good resistance by using open-pollinated seedlings tested with the belt spray method, that also show good resistance in the field.

In *Ecuador*, no significant correlation was found in the % of infected young seedlings tested with the belt-spray method and the % of infected seedlings tested under field conditions. The % of infection of open-pollinated seedlings was not correlated with the level of infection of the clones from where the seeds were obtained.. Furthermore, seedlings that escaped infection with the belt spray method did show similar broom numbers than non-inoculated control seedlings planted side by side in the field. This indicates that the belt-spray method is unreliable for individual seedling selection.

No consistent level of infection was obtained when applying the belt-spray method to inoculate clonal plants, either in *Brazil* or in *Ecuador*. In *Ecuador*, the % of infected growing points obtained on clonal plants was not related to the number of brooms in the field. However, from *Peru* promising results were reported on inoculation of clonal tissue. In *Trinidad*, where large scale testing of clonal plants is carried out with WCF funding, correlations between the % of infection of clonal plants and % vegetative infection in the field are non-significant for individual trees or clones (0.11 to 0.35), but tend to be higher when clones are grouped into classes with different resistance level ( $r = 0.88$  for % of inoculated growing points developing brooms).

### ***Alternative methods***

Recently, the agar-droplet inoculation method was adapted at the UWI in *Trinidad* with the objective to obtain high levels of infection and to measure other resistance parameters, such as the number of days for first symptoms to appear (incubation period: IP) and broom base diameter (BBD). Published results show better correlations with field results for the IP and for BBD than for infection frequency (IF). During the current meeting, additional results were presented by using this method on open-pollinated seedlings obtained from different clones and by using different conditions of inoculation. IF was substantially higher for the agar-droplet method compared to the spray method. Discrimination between clones was best for BBD, good for IP and non-significant for IF. Pruning of seedlings decreased slightly the IP and BBD. Inoculation concentration showed to have no significant effect on IP and BBD. However, the age of the seedlings seems to affect IP, with young seedlings showing symptoms quicker than older seedlings. The best age of inoculation would appear to be between 5 and 8 month old seedlings.

Alternative methods tested in *Brazil* included leaf-disc inoculations and germination tests using sap extracts (from different tissues). Results so far have been inconclusive. The same was observed in *Trinidad* for extracts from different types of tissues. Presently, in *Ecuador* germination of basidiospores on the leaf surface is compared with more traditional methods, including the Holliday test, the belt-spray method and the agar-droplet inoculation method.

### **Pod-resistance to witches' broom**

Relatively little experience has been obtained so far with artificial inoculation of pods for witches' broom resistance. Results obtained in the Amazon region in *Brazil* show however that infection of pods can be obtained with inoculation of pods even older than 3 months. There seems to be an effect of the age of the pod on the resistance level, with more resistant clones developing resistance more rapidly with increased age. In *Bahia, Brazil*, recently inoculation of pods has been initiated and promising results were obtained.

#### **3.4.2 Agreement on proposed working procedures for alternative resistance testing activities methods**

The Working Procedures that have been prepared by interaction between participants, and distributed during the meeting, were discussed. Some changes in these procedures were adopted during the meeting, as reflected in the finalized working procedures. These will be distributed separately to all institutions involved in these activities.

#### **3.4.3 Agreement of collaborative resistance testing activities**

The collaborative activities to be developed within the CFC/ICCO/IPGRI project over the next two years (2005 and 2006) were agreed, using the standardized working procedures as agreed above. The agreed activities are indicated in Table 2, attached to this report.

**TABLE 2. AGREED COLLABORATIVE RESISTANCE TESTING ACTIVITIES**  
 ( for year 1 and 2 of the CFC/ICCO/IPGRI project)

**A. Early screening activities proposed for moniliasis**

Testing method	Costa Rica (CATIE)	Ecuador (INIAP)	Peru (UNAS, ICT)	Venezuela (INIA)
<b>A.1. Preliminary test: inoculation of pre-germinated seeds and seedlings (agar droplet)</b> (Prueba preliminar: inoculacion de semillas pre-germinadas y plántulas)	<b>Pre germ seeds And seedlings</b>	<b>Seedlings only</b>	<b>Pre germ seeds and seedlings</b>	<b>Criollo pre-germinated seed compared with other varieties</b>
<b>A.2. Effect of spore concentration and period of incubation with agar droplet inoculation of seedlings</b> (Efecto de concentracion de inoculo y periodo de incubacion en plantulas inoculadas con gotas de agar)	yes	yes	yes	
<b>A.3. Effect of spore concentration on inoculation of pre-germinated seeds</b> (Efecto de concentracion de inoculo en semillas pre-germinadas)	yes		yes	
<b>B1. Germination of spores on different host tissues</b> (Germinacion de esporos sobre deferentes tejidos de cacao)	yes	yes	yes	
<b>B2. Germination of spores in different plant extracts</b> (Germinacion de esporos en extractos del huésped)		<b>In liquid endo-sperm</b>		
<b>Other activities</b> (otros actividades)				<b>Priority for INIA: pod test applied to Criollo and hybrids</b>

**B. RESISTANCE TESTING METHODS PROPOSED FOR RESISTANCE OF VEGETATIVE PARTS AND PODS TO WITCHES' BROOM, INCLUDING COMPARISON WITH MONILIA RESISTANCE**

<b>Testing method</b>	<b>Brazil (CEPLAC)</b>	<b>Ecuador</b>	<b>Peru</b>	<b>Trinidad</b>
<b>A1. Droplet and spray inoculation of seedlings from open-pollinated and hand-pollinated pods of 6 genotypes</b> (Comparacion de gota de agar y aspercion en inoculacion de semillas de libre polinizacion de 6 clones)	Yes	Yes		Yes
<b>A2. Comparison between sites of clonal resistance to WBD by inoculating open-pollinated seedlings</b> (Comparacion entre paises de resistencia de clones evaluado por plantulas obtenidas por polinizacion libre)	Yes	Yes		Yes
<b>B. Validation of the agar-droplet method for use as an early resistance screening test in pre-breeding</b> (Validacion del metodo de gota de agar para seleccion precoce de plantulas en programas de pre-breeding)	Yes	YES		Yes
<b>C1. Evaluation of pod resistance to WBD by using the standard method for evaluation of resistance to monilia</b> (Evaluacion del metodo standard utilizado para monilia para evaluar resistencia de frutos a escoba de bruja)				Yes
<b>C2. Effect of the age of the fruit</b>	Yes for specific clones	Yes		Yes
<b>C3. Effect of the genotype</b>	5 ICT, 5 local clones	See "D"	See "D"	5 ICT, 5 local clones

## Continuation

Testing method	Brazil (CEPLAC)	Ecuador	Peru	Trinidad
<b>D. Comparison of resistance to moniliasis with that of pod resistance to witches' broom</b> (Comparacion de resistencia a moniliasis y escoba de bruja)		Yes 5 ICT and 5 local clones	Yes	
<b>E. Assessment of witches' broom resistance using germination of spores in plant extracts</b> (Evaluacion de resistencia a escoba de bruja utilizando germinacion de espores en extractos de plantas)	Germination in liquid endosperm			Not a priority
<b>Other methods proposed</b> (Otros metodos propuestos)		Germination on leaves Holliday		

## C. Participation in a ring test for resistance to *Phytophthora* of International Clones to be inoculated by the detached pod method

Testing method	Brazil (CEPEC)	Venezuela (INIA)	Trinidad (CRU/MALMR)
<b>Application of the detached pod test on all clones of the International Clone Trial</b> (Prueba de inoculación de frutas despegadas de todos los clones del ensayo Internacional de Clones)  Note: Pods to be used should come from trees that have been inspected to have similar type of pods and other traits, that correspond with the characteristics of the clone. (Nota: Las frutas deben ser escojidas de arboles que presentan características de frutas y de otros aspectos morfológicos idénticas y que corresponden al las fotos del clon)	Yes, 10 pods per clone	Yes, 10 pods per clone	Yes, 10 pods per clone

**ANNEX 1: FIRST MEETING & PRESENTATIONS pp. 31-333**

**Short and medium-term strategies to avoid frosty pod rot to become a pandemic in Central and South America**

**Monday 21 February**

- 08:00-08:15 Inauguration of the meeting (Pedro Ferreira, Director of CATIE; Martin Gilmour, Industry; Bertus Eskes, IPGRI/CFC).
- 08:15-08:30 Program and brief presentation of participants (Andreas Ebbert).
- 08:30-10:00 Morning Session (Chair P. Hebbar, Masterfoods Beltsville).

**Presentations (10-15 min each)**

- **Harry Evans (CABI, UK):** Frosty Pod Rot - A Review of its basic biology.
- **Wilbert. Phillips (CATIE):** Origin, biogeography and current situation of frosty pod disease in Latin America.
- **Cathie Aime (USDA, Beltsville):** Nomenclature of Frosty Pod Rot Complex - Molecular analysis and impact on breeding.
- **Roy Bateman (Imperial College, UK):** Rational use of control agents against Frosty Pod Rot: how to limit the damage to cacao cultivation with ICM.
- **Allan Pomella (Masterfoods, Brasil):** New Technology to Monitor FPR - Applied Research in Brazil.

10:30-12:00 Continuation of the morning session (10-15 min each)

- **Eduardo Somarriba (CATIE):** Cocoa pod losses due to monilia: shade species, inter-clonal crosses and spore movement in Talamanca, Costa Rica.
- **Enrique Arévalo (Instituto Cultivos Trop., Perú):** Current Situation in Peru and farmer outreach programs.
- **Carmen Suárez (INIAP-Ecuador):** Current Situation in Ecuador and methods of control -Biological/Conventional
- **Rolando Ríos (UNAS-Peru):** Epidemiología y zonas de riesgo de introducción de monilia a Brasil y Bolivia.
- **Belize:** Impact and control of moniliasis in Belize.

13:30-15:00 Round Table Discussions.

15:30-17:30 Break out groups: Epidemiology, IPM, Molecular Biology, Farmer, outreach-training, Quarantine issues, etc.

**Tuesday 22 February**

- 8:30-12.00 Plan of Action for Belize/Mexico/Brazil/Bolivia - next steps
- 13:30-17:00 Continuation.

**SECOND MEETING & PRESENTATIONS pp. 334-753**

**American Cacao Breeding Program, planning and discussion meeting.**

**Wednesday 23 February, 2005**

**Session 1. Introduction/National Cocoa Breeding Programs (priorities)**

**Moderator: Bertus Eskes, Secretary David Butler**

- |                |  |
|----------------|--|
| 8:00-8:10 am   | Recall on discussions held at University of Reading in 2004 concerning the different Regional Cocoa Breeding Programs.<br><b>Roger Dehnel</b>  |
| 8:10-8:20 am   | Update on the discussions held until now regarding the Latin American Cocoa Breeding Program<br><b>Juan Carlos Motamayor</b>   |
| 8:15-9:15 am   | Short presentations on National Cocoa Breeding Programs (descriptions of short, medium and long term objectives) with special emphasis on the activities that could benefit from the Latin American Breeding Program. <ul style="list-style-type: none"> <li>• Angel Castillo, Venezuela (10 minutes)</li> <li>• Luis Garcia, Peru (10 minutes)</li> <li>• Maharaj Kamaldeo, Trinidad (10 minutes)</li> <li>• Wilbert Phillips, Costa Rica (10 minutes)</li> <li>• Jesus Sanchez, Honduras (10 minutes)</li> <li>• Alejandro Lopez, Mexico (10 minutes)</li> </ul> |
| 9:15-9:45 am   | Questions and Answers  |
| 10:15-10:45 am | Cont. Short presentation <ul style="list-style-type: none"> <li>• Uilson Lopez, Bahia, Brazil (10 minutes)</li> <li>• Jay Wallace, Belem, Brazil (10 minutes)</li> <li>• Freddy Amores, Ecuador (10 minutes)</li> </ul>  |
| 10:45-11:00 am | Questions and Answers  |

## **Session 2. Review of cocoa breeding activities performed at the regional level**

**Moderator Martin Gilmour, Secretary Carlos Astorga**

11:00 am-11:45 am Review of the USDA/Masterfoods Cocoa Breeding Program in Latin America and other Regional Activities.

- Introduction (Juan Carlos Motamayor, 10 minutes)
- Activities in Ecuador (Geover Peña, 15 minutes)
- Activities in Costa Rica (Wilbert Phillips, 15 minutes)

11:45 am-12:00 pm Questions and answers

1:30-2:15 pm Cont. of presentations

- CFC cocoa breeding program (Bertus Eskes, 15 minutes)
- Activities in Brazil USDA+CFC (Uilson Lopez/Didier Clement, 15 minutes)
- Activities at CRU (David Butler, David Iwaro, 15 minutes)

2:15-2:30 pm Questions and Answer

2:50-5:00 pm Revision\Prioritization (justification) of the previously discussed objectives\goals for the Latin American Breeding Program. Strategies\methodologies to achieve the goals identified.

**Thursday 24 of February (8:00 am-12:00 pm).**

## **Session 3. Open discussion**

**Moderator Juan Carlos Motamayor, Secretary David Iwaro.**

- Identification of individuals/institutes responsible for carrying out the activities identified as of priority for the Latin-American Cocoa Breeding initiative.
- Identification of contributors to write the program.
- Identification of steps to get funding for the program.

**THIRD MEETING & PRESENTATIONS pp. 754- 1076.**

**Resistance Testing Method Meeting**

**Thursday 24 February (13:30 to 17:30)**

13:30-13:40 Rationale and objectives (Bertus Eskes, IPGRI)

**Session 1. Monilia resistance testing methods**

*Chairman: Freddy Amores, Secretary: Rolando Rios*

13:40-15:00 Presentations on correlations with field resistance, problems faced and solutions tried, research results on alternative pod inoculation methods, proposed future activities on alternative resistance testing methods (presentations of 10 min, followed by 5 min discussion):

- Wilbert Phillips y Carmen Garcia, CATIE, Costa Rica
- Carmen Suarez and Karina Solis, INIAP, Ecuador
- Rolando Rios and Jaime Chavez, UNAS, Peru
- Enrique Arevalo, Peru
- Raisa Rumbos, INIA, Venezuela

15:00-15:30 Results obtained with inoculation of seeds or seedlings

- Harry Evans, CABI, UK
- Other results?

16:00-16:30 Agreement on proposed working procedures for early resistance testing

16:30-17:00 Agreement on activities for the next two years

**Friday 25 February (8:00-12:30)**

**Session 2. Resistance to Phytophthora pod rot**

*Chairman: David Butler, Secretary: Jean-Marc Thèvenin*

08:00-8:45 Correlation of leaf disc and pod inoculations with field results, problems faced (10 min presentations with 5 min discussion).

- Edna Luz and Stela Dalva Silva, CEPLAC, Brazil
- David Iwaro and Jean-Marc Thèvenin, CRU/CIRAD, Trinidad
- Karol Alpízar and Wilbert Phillips, Costa Rica

08:45-09:00 Agreement on proposal on detached pod inoculations of International Clones (Brazil, Venezuela, Trinidad,..)

### **Session 3. Resistance of vegetative parts to witches' broom**

Chairman: Uilson Lopes (or Wilson Monteiro), Secretary: Juan-Carlos Motamayor

09:00-10:30 Presentations on correlations with field resistance, problems faced and solutions tried with seedling and clonal materials, results on alternative or improved methods, proposed future activities on resistance testing methods (presentations of 10 min, followed by 5 min discussion):

- Edna Luz and Stela Dalva Silva, CEPLAC, Brazil
- Carmen Suarez and Ricardo Delgado, INIAP, Ecuador
- Rolando Rios and Jaime Chavez, UNAS, Peru
- Jean-Marc Thèvenin, CRU/CIRAD, Trinidad
- Dercy Parra, INIA, Venezuela

11:00-12:00 Agreement on proposed Working Procedures for **early resistance** testing of seedlings and clones

12:00-12:30 Agreement on activities for the next two years

### **Friday 25 February (13:30-17:00)**

### **Session 4. Pod resistance to witches' broom and comparison to monilia resistance**

*Chairman: Rolando Rios, Secretary: Enrique Arevalo*

13:30-14:20 Proposals for studies on pod resistance to witches' broom and comparison with monilia resistance (10 min each)

- Edna Luz and Stela Dalva Silva, CEPLAC, Brazil
- Carmen Suarez and Karina Solis, INIAP, Ecuador
- Rolando Rios and Jaime Chavez, UNAS, Peru
- Jean-Marc Thèvenin, CRU/CIRAD, Trinidad
- Others ? (Raisa Rumbos, INIA, Venezuela?)

14:20-15:00 Agreement on proposed Working Procedures for pod resistance testing for witches' broom and comparison with monilia resistance

15:00-15:30 Agreement on activities for the next two years

### **CONCLUSIONS AND CLOSING SESSION (OF ALL THREE MEETINGS)**

16:00-16:45 Presentation of conclusions and recommendations

- Monilia Alert meeting
- Regional Breeding Meeting
- Resistance Testing Methods Meeting

16:45-17:00 Closing session (informal)

## ANNEX 2. LIST OF PARTICIPANTS

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