BetoCarib

Begomovirus Disease Management for Sustainable Production of Tomato in the Caribbean

Fifth Framework Programme

Confirming the International Role of Community Research

Begomovirus disease, transmitted by the white-fly Bemisia tabaci is responsible for important economic losses in tomato crops in the Caribbean.

To ensure sustainable development, disease management strategies must be multi-component in nature and based on a thorough understanding of the pathosystems involved, because of the limited resources of growers, the diversity of agrosystems and the fragility of ecosystems within the region.

BETOCARIB aims to study Bemisia-begomovirus-host plant pathosystems and their epidemiology to develop models in order to identify the key factors involved in the propagation of the disease. Integrated pest management strategies able to reduce the impact of the disease on tomato crops will be developed from these models and tested within the various islands. The most effective one will be transferred and adapted to the whole islands to achieve the sustainable management of begomovirus disease. This will be done through scientific and technological cooperation between three the European and five Caribbean research teams.

Project Information
Partners
Project Members Only

The project started in 2002 and closed in 2007.

The results are available on the next page 'Project information'. More specific informations may be asked to the partners of the project.

The European Commission **Community Research**

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BEgomovirus diseases management for sustainable production of TOmato in the CARIBbean

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ABSTRACT

Begomovirus diseases transmitted by Bemisia tabaci are responsible for important economic losses in tomato crops in the Caribbean islands. Because of the limited resources of growers, the diversity of the agro-system and the fragility of the ecosystem, the management strategies must be multi component in nature and based on a thorough understanding of the pathosystem involved to ensure sustainable development.Tin thois context, the general objective of BETOCARIB is to develop integrated pest management (IPM) packages adapted to the fragile insular ecosystems, including low pesticides application and resistant varieties, to increase tomato production and to ensure the sustainability of cropping systems both in large and small-scale growing areas of the Caribbean islands where the whitefly-transmitted begomoviruses threaten the tomato crops. This was achieved through : (i)WPA an increasement of scientific knowledge on epidemiology of viruses transmitted by whiteflies in tropical areas by combining the expertise of European and Third countries research groups, (ii)WPB the identification of the key factors involved in epidemics and the development of epidemiological models, based on these model (iv)WPC the establishment of IPM packages adapted to growers with small plots and limited resources in the context of year round tomato production.

The begomovirus statute is now updated in the Caribbean islands. The presence of TYLCV is clearly confirmed as dominant both for Cuba and Dominican Republic even mixed infection by ToHMV could occur in Cuba. Martinique and Guadeloupe have characterized TYLCV as a recent introduced begomovirus in addition to PYMV already identified. At present both viruses exist with high incidences in tomato production areas. Trinidad and Tobago have confirmed the presence of a new species of begomovirus characterized as PYMTV. Several molecular tools (primers and probes) were developed for the diagnosis of each virus. Hybridization techniques were found to be well adapted for epidemiological studies, immunocapture PCR was performed to detect viruses in weeds and facilitate the identification of weed sources. Finally based on sequence alignment, new set of degenerate primers were designed to theoretically detect all begomoviruses and to distinguish either TYLCV from other bipartite begomoviruses or old world begomoviruses from new world ones. The hybridization technique using non radioactive probes is chosen as the unique common protocol to detect begomoviruses during the project.

Bemisia tabaci samples were collected both in tomato and wild host in different agro ecological zones. Biotype B of B. tabaci was the only one dominant in any zones. It was characterized using RAPDs; microsatellite markers developed during the project and sequencing of a part of the cytochrome C zone.

Due to contrasted situations existing in the Caribbean islands, a comprehensive list of variables important in epidemic was identified to provide a "maximal model" using General Linear Modelling approaches as the basis for the analysis of the data collected during surveys. The first epidemic data collected from Guadeloupe was highly informative to adapt the 'maximal model' proposed in order to define an ecosystem-based begomovirus disease risk prediction model. Protocols developed in Guadeloupe are transferred and adapted to Cuba to test during the third year the hardiness of the keys factors suspected involved in the epidemics.

Predictions of *B. tabaci* seasonal population fluctuations have been possible using climatic and natural enemy data. Cloth barriers to reduce whitefly entry to tomato crops have been evaluated under a variety of conditions. A model of epidemiology of begomovirus disease progress and *Bemisia tabaci* population dynamics within the tomato crop has been developed which provides a good fit to three pairs of experimental data, allowing new understanding about the effects of control interventions. Extensive epidemiological studies using disease surveys have shown that Imidacloprid use, physical protection of the crop and a variety of good general tomato husbandry practices were associated with low begomovirus disease in both Guadeloupe and Cuba.

In parallel, Cuba, Martinique and Trinidad set up several trials to screen tomato varieties identified as promising in different Caribbean islands for their resistance to TYLCV and PYMV as well as resistance to nematodes and high temperatures. Tomato cultivars resistant to different begomoviruses and adapted to tropical conditions were identified and evaluated in IPM trials. Two IPM packages were defined for Cuba and French West Indies and tested in terms of disease control and economic impact according to the final objective to reduce begomovirus losses in tomato crop. A combination of different factors was assessed with regards to environmental conditions of producing areas:

The "traditional practices + susceptible variety" lets a high viral severity which had a negative influence on tomato productivity. The "traditional practices + resistant variety" and "IPM practices with a resistant variety" sygnificantly contributed to avoid damages. These results indicate that using a resistant variety in a correct IPM package bring a solution to grow tomatoes under begomoviruses infected areas. Several technical sheets for the growers were co-edited in 2005 by CIRAD, National Chamber of Agriculture, Plant protection unit of the ministery of agriculture, cooperative sOCPAM and the regional federation of crop protection against noxious organisms.

At the beginning of the project, a BETOCARIB Web site (htpp://betocarib.cirad.fr) to formalise the interactions between consortium members and to keep us aware on the project progress was created. Due to these objectives, the Betocarib Web site has been divided into two parts: everybody can consult one and the other one is restricted to members only.

SUMMARY OF FINAL REPORT

The general objective of BETOCARIB is to develop integrated pest management (IPM) packages adapted to the fragile insular ecosystems, including low pesticides application and resistant varieties, to increase tomato production and to ensure the sustainability of cropping systems both in large and small-scale growing areas of the Caribbean islands where the whitefly-transmitted begomoviruses threaten the tomato crops. This project has been achieved through the three following work packages. WP A was devoted to the identification of begomoviruses, their relative incidence, their vectors and their respective host plants in order to develop specific tools for their detection. WP B was focused on epidemiology studies in order to build an adequate agronomic model to predict begomovirus infestations. WP C contributed to define adequate IPM strategies to control viruses based on the use of tolerant/resistant varieties, the cultural methods and prediction of infections due to the agronomic epidemic model developed.
Results achieved were presented as deliverables WP by WP.

WP A - IDENTIFICATION OF BEGOMOVIRUSES AND THEIR VECTORS AFFECTING TOMATO CROPS IN THE CARIBBEAN ISLANDS AND DEVELOPMENT OF TOOLS FOR THEIR SPECIFIC DIAGNOSIS

D1 - **Estimation of the diversity of begomoviruses and vectors affecting tomato/ publication**

BEGOMOVIRUSES

➔ Cuba

Different begomoviruses were identified on tomato in Cuba viz TYLCV (dominant) and ToMHV, during the early stages of the project. The latter has however been completely displaced in tomato and TYLCV is presently the only virus affecting tomato at high incidences.
Several isolates of TYLCV were collected from diverse areas during the project period and only one TYLCV strain was prevalent.

➔ Dominican Republic

TYLCV is the dominant begomovirus infecting tomato in DR. Another uncharacterized begomovirus was identified in some tomato samples. The incidences are high in locations where processing tomato is grown (large contiguous areas), but is low where salad tomato is grown (fragmented locations)

➔ Guadeloupe and Martinique

PYMV was the only begomovirus present at the start of the project. One year later TYLCV was identified in both Guadeloupe and Martinique. At present both viruses exist, with high incidences in tomato production areas.

➔ Trinidad and Tobago

PYMTV was the only begomovirus confirmed present in Trinidad and Tobago during the project period. This virus is found at high incidence in all tomato growing areas. This virus was originally described as a strain of PYMV but was later designated as a new species by Faquet and Stanley (2003): Annals of Applied Biology.

WHITEFLIES VECTORS

Bemisia tabaci was collected from different agro ecological zones in tomato and other bemisia hosts, both wild and cultivated. Biotype B of *Bemisia tabaci* was the most dominant whitefly biotype found throughout the Caribbean. Only two samples, one collected from Guadeloupe and one from Martinique were identified as biotype-A. These however were collected from *Euphorbia* and sweet potato respectively.

Transmission tests

Tomato to tomato transmission tests were carried out on TYLCV and PYMV using 3, 10 and 25 adults. TYLCV was more efficiently transmitted to tomato than PYMV (Transmission efficiency was 60% for PYMV and 100% for TYLCV using 25 adults per plant). This was not carried out for PYMTV.

D2 - **Specific tools useful for the development of epidemiological studies and their use in the determination of the prevalence of begomoviruses in the Caribbean.**

- ➔ Specific molecular probes were developed for each begomovirus and were found to be useful for epidemiological studies. These were used as common tools in all epidemiological studies developed afterwards.
- ➔ Immunocapture-PCR was developed for the detection of begomoviruses in weeds and was used in the identification of weed sources.

- Based on the updated sequence database of begomoviruses, new degenerate primers were designed to theoretically detect all begomoviruses. Another set of degenerate primers to distinguish TYLCV from other bipartite begomoviruses and to distinguish old world begomoviruses from new world begomoviruses were developed (Ref year-1 report).

These degenerate primers need further validation before universally accepted.
These tools were used to detect the prevalence and incidence of tomato infecting begomoviruses in the various islands during the project.

D3 - **Identification of weeds as sources of pathogenic begomovirus for tomato crops/publication**

A list of known begomovirus host species (weed and cultivated) was compiled based on the literature for each begomovirus. These and species found around and in tomato crops were tested for the presence of tomato begomoviruses.
However, transmission tests to confirm the identified begomovirus hosts as potential virus sources to tomato crops were not carried out.
The survey however identified a number of non-tomato infecting begomoviruses in weeds. eg. In Cuba: Jatropha mosaic virus in Jatropha gossipyfolia, Macroptylium mosaic virus in Pseudelephytopus spicatus. In Guadeloupe: Jatropha mosaic virus was detected in Jatropha gossipyfolia. Other distantly related begomoviruses to known begomoviruses were detected in Rhyncosia, Euphorbia, Merrimia, Corchorus, Sida and Malva species. In Trinidad and Tobago: begomoviruses closely related to Calopogonium mosaic virus, Rhycosia mosaic virus and Sida golden mosaic virus were detected in Rhycosia, Calopogonium and Sida species.

→ TYLCV

Cuba

A list of TYLCV hosts was published by Gonzalez (1995). TYLCV was detected for the first time in beans, pepper, squash and Euphorbia during the project period.

Dominican Republic

Since it was extensively dealt with in another study by Salati et al (2002) Phytopathology 92 (5): 487-496, this was not pursued in the present study.

Guadeloupe and Martinique

Of the species tested TYLCV was found only in bean. It was not detected in any of the listed TYLCV host species.

→ PYMV

PYMV was only detected in pepper in Guadeloupe. None of other reported species were infected with PYMV.

→ PYMTV

An exhaustive list of weed and host species were surveyed for the first time. PYMTV was only detected in pepper and at very low frequencies in Trinidad and Tobago.

D4 - **Overview of the ecological conditions in the tomato growing areas**

During the first workshop in Guadeloupe the various Caribbean islands were divided into appropriate agro-ecological zones for sampling purposes and epidemiological studies.

D5 - **Training session**

Training of DNA isolation from weeds and immunocapture-PCR for detection of begomoviruses from weeds were conducted in Trinidad. Additionally the newly developed degenerate primers were tested for their efficacy using various begomoviruses found in the region.

WP B - EPIDEMIOLOGY AND MODELLING TO INVESTIGATE THE APPROPRIATE MIX OF DISEASE CONTROL TOOLS AT BOTH CROP AND ECOSYSTEM LEVEL

D6 - **Ecosystem based begomovirus disease risk prediction for the Caribbean islands/publication**

Basse Terre & Grand Terre:

- At extreme values, ***whitefly abundance*** appeared to have an impact on disease. For the majority of cases with more intermediate values, whitefly abundance was not a good predictor of disease. Any control measures directed at whiteflies would need to be able to reduce whitefly numbers to

very low levels before they would be likely to have ***any significant impact*** on disease incidence.

- ***Physical protection of the crop*** was also an important variable. This was scored as follows: 1 if a greenhouse was entirely enclosed by plastic, glass or insect-proof net or the plot had well developed barrier rows; 2. if the greenhouse was not fully enclosed or the barrier rows were less well developed; and 3. if no form of physical protection was used. Those fields with the ***highest physical protection score always had the highest score for management practices***. Physical protection was also carried out in nurseries in some cases and there was a link between the use of physical protection in field and nursery. For those fields ***were the highest level of physical protection was used in the field, it was also used in the nursery***.
- Requiring further investigation was an interesting ***inverse relationship between physical protection and chemical use***. Those fields with the highest chemical use score, all had the lowest physical protection score. Chemical use was scored not only according to use, but also according to whether the insecticide was used correctly. Thus a score of 3 indicates proper use of pesticides. The results suggested that physical protection and chemical use represented alternative approaches used by farmers to begomovirus disease control.
- The results show that three variables: ***good general management practices***, ***physical protection in field*** and ***physical protection in nursery*** represent a package of measures which together were associated with low disease incidence. Although ***chemical protection*** had some association with lower incidence, its ***effect was quite weak***. This may be partly due to the fact that chemical use appeared to be used as an alternative to the package of measures which was strongly linked to low disease incidence. The result suggests a need to clarify the impact of whitefly insecticides on Begomovirus disease incidence under controlled conditions.

CUBA:

- ***Regions*** were distinguished as Western, Eastern and Central. Fields in the western region were 6 times less likely to be infected with begomovirus than fields in the eastern region. The 95% confidence intervals were wide, ranging from 1.2 to 31 times less likely. Central region fields were not significantly different from the Eastern Region.
- ***Oct to Dec planting*** was 25 times less likely to suffer infection than Feb to July planting.
- Fields with a ***slope*** (as opposed to a lack of slope) were less likely to be infected but the effect was small and the reason is unknown.
- There was a suggestion that fields with ***barriers*** to *Bemisia* movement tended to be less likely to be infected but the effect was not significant.
- ***Sheltered cultivation*** however had a massive effective with infection being 39 times less likely than open field cultivation.
- Higher scores for **Bemisia *abundance*** incurred higher infection risk, the effect being significant but not very large.
- By far the most important contributing factor to infection risk was ***varietal resistance***, with those varieties classified as resistant being 100 times less likely to exhibit begomovirus infection.
- If ***planting*** occurred in the ***later*** rather than the earlier months of either planting season there was an increased risk (3.8 times) of infection.
- Overall, the model predicted ***infected fields correctly 85%*** of the time (110/130 cases) and predicted ***uninfected fields correctly 90%*** of the time (85/94 cases).
- Certain variables tended to be associated with each other, i.e. particular sets of conditions tended to occur in the same field. Ten of the variables tended to be linked in the same fields, i.e. a field either had generally high scores or generally low scores for this set of variables. These variables were: ***field physical protection, field sheltered cultivation, general management practices, varietal resistance, growing season, field chemical protection, previous virus hosts, host continuity, nursery physical protection, and nursery chemical protection***. High scores for this set of variables appear to represent a package of physical and chemical protection of the crop in nursery and field, use of resistant varieties and good general management practices. Linked to this were high scores for *Bemisia* host continuity and previous virus hosts, suggesting this cluster represents fields of well-managed but intensive tomato production. High scores for these variables (i.e. Cluster 1) were associated with low begomovirus disease incidence and severity. 76% (14/58) of fields in Cluster 1 were not infected whereas 73% (113/155) of fields in Cluster 2 were infected. 94% (65/69) of fields in Cluster 1 had disease severity scores of 1 or less whereas 61% (94/155) of fields in Cluster 2 has severity scores greater than 1. Clearly, therefore a group of variables was associated with begomovirus disease and because this group were correlated it was not possible to say which were of particular importance. The logistic regression highlighted sheltered cultivation and varietal resistance and these two variables gave the best model.

However these variables were interchangeable with other members of the ten listed above to give alternative models which had were nearly as good.

D7 - **Model of the epidemiology of begomovirus disease progress and *Bemisia tabaci* population dynamics within the tomato crops/publication**

- A ***crop model*** was developed which represents whitefly dynamics and begomovirus infection in a single tomato plot. A set of linked differential equations specifies the model. Initially, all plants are healthy; during the course of the simulation they become infected at a rate depending on the number of infective whiteflies and the inoculation rate parameter. A corresponding term describes how whiteflies acquire infection, dependent on the number of infected plants and the acquisition rate parameter.
- The model was used to investigate the likely impact of ***management interventions***. For example, in plots protected by cloth barriers, immigration of whiteflies is reduced. Immigration rate is one of the model parameters and so the relationship between immigration rate and disease progress in the crop can be explored using the model.
- In two experiments carried out in Guadeloupe ***barriers*** were used to reduce the entry of the virus vector, *Bemisia tabaci*, to tomato plots. The barriers erected around the crop were of insect-proof cloth fences 1.5m in height, in one case with an insecticide-treated, insect-attracting strip facing inwards.
- The two viruses present, tomato yellow leaf curl and potato yellow mottle, had similar disease progress curves and the model was fitted to the symptom data from the treated and control (unprotected) tomato plots.
- ***Parameter estimates*** so obtained indicated that the ***barriers reduced vector immigration by approximately 12-fold*** but that *B. tabaci* retention within the plots was also increased slightly despite the mortality caused by the insecticide-treated strips.
- Actual and simulated disease establishment was delayed by approximately two weeks.
- Results from the other experiment involving ***barriers deployed without insecticide-treated strips*** could be explained by a large increase in *B. tabaci* retention within the barriers resulting in more rapid virus disease progress than in controls.
- The crop modelling indicates that ***partial insect barriers can be worse than none*** because sufficient whiteflies can enter to establish a population and at the same time large numbers are retained in the barrier plot so increasing population growth.

D8 - Prediction of mix of control measure efficiency

- Resistant varieties, if available.
- Field use of whitefly insecticides / biocides especially for partially resistant varieties.
- Physical and possibly, chemical protection of nursery especially in conditions of high inoculum pressure.
- Use of 'open sided' sheltered cultivation in rainy season (Cuba).
- Avoid closed sheltered systems in which whiteflies can be trapped.
- Use whitefly traps if closed sheltered systems are used.
- Grow tomato crops in small isolated vegetable systems separated by barriers of non-Bemisia, non-virus hosts.
- Encourage crop free periods where possible
- Avoid planting late in the season relative to other growers.
- Year-round intensive tomato systems may be sustainable provided a high level of virus disease management is maintained including the growing of resistant varieties.
- Effective irrigation (drip irrigation, Guadeloupe), diligent weeding (?) and use of strong healthy plantlets reduces disease severity to some extent.

D9 - **Design of IPM trials from D8**

(i) *Evaluation of component technologies*

Two key variables that were negatively associated with begomovirus disease incidence and whitefly abundance, ***barriers and chemical protection***, were evaluated in management trials in Guadeloupe and Cuba, respectively.

As explained under D7, the results of three barrier trials in Guadeloupe indicated that whitefly proof cloth barriers that had inward-facing insecticide treated strips proved effective in slowing down the onset of disease by about two weeks. Without the insecticide strips, disease progress was actually faster than

controls under this treatment. Therefore, ***barriers, including different types of screened cultivation systems, can in some circumstances retain whiteflies and exacerbate disease.***
Insecticide trials in Cuba evaluating the effectiveness of applying ***imidacloprid in the nursery*** and in the field showed that standard farmer practices were not effective in reducing whitefly numbers or disease incidence. The implications of the results are that ***seed treatment should be combined with physical protection in the nursery and earlier application of imidacloprid in the field than is commonly done***.

(ii) *Development of IPM packages*
In both the greater and lesser Antilles (Cuba and Guadeloupe/Martinique, respectively), ***resistant varieties*** were chosen as the first component of an IPM strategy. The ***agronomic and requirements disease resistance characteristics of the varieties differ between locations and seasons***. For example, resistance to *Ralstonia solanacearum* is required in Martinique, Guadeloupe and in certain locations in Trinidad whereas it is not needed in Cuba.

WP C - EVALUATION OF IPM PACKAGES BASED ON VROP MANAGEMENT AND RESISTANT VARIETIES

D10 - **Identification of tomato cultivars resistant to different begomoviruses and other constraints**

Twelve tomato cultivars, either released by private companies (Hazera) and by Cuban national breeding program, were identified as promising in different Caribbean islands participating in the project according to the trial results obtained in agricultural conditions. The table below shows the cultivars recommended by each partner based on their resistance to begomoviruses and other specific constraints.

Country	Cultivar	Recommendation	Other considerations
Cuba	Vyta	Open field (optimum and late season)	Heat tolerant
	C7, C12, C35, C48	Shelter cultivation (optimum season)	Heat tolerant, C12 resistant to nematodes
	C41, C48	Shelter cultivation (off season)	Heat tolerant, C48 yield stable
Martinique	Vyta	Open field ; - during dry season : no grafting recommended - hot wet season: mandatory grafting on a BW-resistant variety	Very susceptible to bacterial wilt
	C48	Open field ; - during dry season : no grafting recommended - hot wet season: mandatory grafting on a BW-resistant variety	Good yield (2003, 2004) Susceptibility to BW: tested in July 2006
	HA3048, HA3017	HA3048 = best yield in 2003, and best virus tolerance	
Trinidad and Tobago	HA3018, C57	Open field (both early wet and late wet)	HA3018 is immune to PYMTV, C57 shows low begomovirus incidence, low severity score, stable yields; both susceptible to bacterial wilt.

	HA3105,HA3108, C48, C12	Open Field (Early wet season)	Low begomovirus incidence, low severity score, stable yields; All susceptibility to bacterial wilt.

➔ In Cuba, 5 cultivars were recommended, not only for open field but also for sheltered production. In this country, begomoviruses resistance and heat tolerance are compulsory.
➔ In Martinique, main constraints apart of begomoviruses are heat and bacterial wilt. Two main varieties were recommended, but only in the season when bacterial wilt pressure is not too high. For the rest of the year, grafting on a BW-resistant line is mandatory.
➔ In Trinidad, 5 cultivars were recommended according to the local constraints. We can notice that C48 and Vyta were recommended in islands where different viruses (TYLCV, PYMV, PYMTV) were prevalent. These varieties may carry a broad resistance that should be preferred as a component of durability in the project.

D11 - Complete IPM packages according to ecosystems and market

Country	Recommended IPM	Market
Cuba	Resistant varieties Insect proof nursery to tolerant varieties Verticillium lecanii to young Bemisia control	
Martinique	1. Varieties with combined resistance = not available (INRA cv. expected in 2008?) At the moment, advice = crop Vyta during the dry season in BW-free areas	Fresh market only
	2. Crop free period of 3 months in the North Leeward Coast. => implies the involvement of growers, to stop collectively the production, and transfer it to South	Big round red fruits only
	3. Grow plants in insect-proof nurseries : possible only in cooler areas : central Hills of the island	
	4. use Vyta grafted on a BW-resistant rootstock (e.g. Caraibo) during the hot season - cost constraints (manpower) and nurserists' skills to be improved	
Trinidad and Tobago	IPM measures have been shown to be not useful with highly resistant varieties and highly susceptible varieties. However, they were found to be important with moderately resistant varieties. The following are specific IPM packages for two of the most important tomato growing areas. **Aranguez** Crop free period during the wet season Synchronous planting of crop earlier Use highly resistant varieties Nursery Chemical Protection Nursery Physical Protection **Bonne Aventure** Crop free period during the dry season Use highly resistant varieties Nursery Chemical Protection Nursery Physical Protection Field Chemical Protection Use of barriers	Fresh market only

Future recommendation:

➔ The selected varieties in each location must be subjected to multiseasonal, multilocational and farmer field trials prior to release in the countries.

➔ Bulletins and extension leaflets developed to provide information and guidance to farmers.

➔ Farmer demonstrations have been done to popularize the varieties.

➔ Partner-7 has developed systems to mass produce seeds of tomato cultivars developed in Cuba for cultivation in the region. Further work is being done to pyramid genes for resistance, through molecular marker assisted selection, provide greater stability against virus evolution.

➔ Relationship between regional suppliers of seeds has been made to market tomato seeds throughout the region.

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CONSOLIDATED SCIENTIFIC REPORT

Objectives

The general objective of BETOCARIB is to develop integrated pest management (IPM) packages adapted to the fragile insular ecosystems, including low pesticides application and resistant varieties, to increase tomato production and to ensure the sustainability of cropping systems both in large and small-scale growing areas of the Caribbean islands where the whitefly-transmitted begomoviruses threaten the tomato crops. This will be achieved through : (i) an increasement of scientific knowledge on epidemiology of viruses transmitted by whiteflies in tropical areas by combining the expertise of European and Third countries research groups, (ii) the identification of the key factors involved in epidemics and the development of epidemiological models, based on these model (iv) the establishment of IPM packages adapted to growers with small plots and limited resources in the context of year round tomato production. Finally, it is expected through this project a scientific and technical co-operation between the research institutions within the community and the Caribbean research institutions (since the Lesser Antilles are not often involved in major projects concerning the Caribbean area).

Specific Scientific and Technological Objectives

The proposed project covers 5 scientific research fields: biosystematics, epidemiology, plant screening, modelling and agricultural practices, inter acting each other to contribute to the development of IPM packages for begomoviruses management in tomato crops including risk management.

Biosystematics

- To characterise the begomoviruses and whiteflies involved in tomato crops in the five Caribbean islands.
- To develop specific tools for the diagnosis of the different begomoviruses identified.

Epidemiology

- To evaluate the incidence of the begomoviruses, according to the different cropping areas.
- To identify the inoculum sources (vectors and weeds) of the different begomoviruses.
- To identify the key-factors influencing the development of epidemics, in relation to agroenvironmental conditions.

Modelling

- To build an inoculum pressure model representative of the different agroecosystems.
- To build an epidemiological model at the crop level and test it in situ.

Integrated pest management

- To screen tomato varieties resistant to the major begomovirus diseases, and adapted to other main pathogens of the area.
- To design IPM packages including resistant varieties with regards to relevant data from epidemiological models.
- To evaluate the IPM packages *in situ*.
-

Results achieved

Results achieved were presented as tasks WP by WP.

WP A - Identification of begomoviruses and their vectors affecting tomato crops in the Caribbean islands and development of tools for their specific diagnosis

T01.01 - Workshop Wo1 and sampling in 5 islands (tomato, weeds and insects)

The first workshop was held from 4th to 8th March 2001 in Guadeloupe (FWI) with specific objectives to standardize the methodology of the survey and clarify each task of the first year of BETOCARIB.

Two invited scientists, Dr Judith Brown (Tucson University –Arizona) and Dr Douglas Maxwell (Wisconsin University) also joined this meeting. Their input were highly beneficial, especially regarding characterization of both vector "Bemisia tabaci" and virus by sharing their experiences on the begomoviruses surveys in vegetables in Central and Latin America, and in Africa. Their presence helped us to define the best-appropriated ways to adopt.

Technique, procedures and protocols and details to perform tasks were stated during the workshop. A first report was written by the co-ordinator of the WPA, partner P1, send to each partner and available on the BETOCARIB web site from the part restricted to the partners only.

Each Partner left the meeting with a clear idea of what has to be done exactly to reach maximal efficient.

T01.02 - Identification of begomovirus infecting tomato

The viral statute is established for each 5 islands using a methodoly defined during the first workshop (see annexes). The survey was exhaustive with the aim to select 50samples representing the diversity of the viral situation. The identification of viruses was realised by adapted molecular techniques faciliting the distinction between both bipartite an monopartite begomoviruses.

➔ Cuba

Different begomoviruses were identified on tomato in Cuba viz TYLCV (dominant) and ToMHV, during the early stages of the project. The latter has however been completely displaced in tomato and TYLCV is presently the only virus affecting tomato at high incidences.

Several isolates of TYLCV were collected from diverse areas during the project period and only one TYLCV strain was prevalent.

➔ Dominican Republic

TYLCV is the dominant begomovirus infecting tomato in DR. Another uncharacterized begomovirus was identified in some tomato samples (Ref report year-2). The incidences are high in locations where processing tomato is grown (large contiguous areas), but is low where salad tomato is grown (fragmented locations)

➔ Guadeloupe and Martinique

PYMV was the only begomovirus present at the start of the project. One year later TYLCV was identified in both Guadeloupe and Martinique. At present both viruses exist, with high incidences in tomato production areas.

➔ Trinidad and Tobago

PYMTV was the only begomovirus confirmed present in Trinidad and Tobago during the project period. This virus is found at high incidence in all tomato growing areas. This virus was originally described as a strain of PYMV but was later designated as a new species by Faquet and Stanley (2003): Annals of Applied Biology (ref.).

T01.03 - Identification of Bemisia tabaci biotype

The presence of *Bemisia tabaci* was reported in all epidemiological contexts from different agro ecological zones in tomato and other bemisia hosts, both wild and cultivated. The characterization of the biotype was performed using two different approaches: using microsatellites markers to distinguish between B and non B individuals and sequencing of an amplified fragment of cytochrome oxidase 1gene (CO1). Biotype B of *Bemisia tabaci* was the most dominant whitefly biotype found throughout the Caribbean.

T01.04 - Development of tools for diagnosis of the identified virus if not available

During the Wo1 the diagnosis by total DNA plant extract hybridization with PCR labelled probe with AlkPhos system was statute as the most enabled technique to treat easily a lot of samples and a commun protocol was defined to be use during the project for the epidemiological studies (see annexes). Immunocapture-PCR was developed for the detection of begomoviruses in weeds and was used in the identification of weed sources. This technique was optimised during the laboratory session held in Trinidad and Tobago during the first meeting (see annexes). Based on the updated sequence database of begomoviruses, new degenerate primers were designed to theoretically detect all begomoviruses. Another set of degenerate primers to distinguish TYLCV from other bipartite begomoviruses and to distinguish old world begomoviruses from new world begomoviruses were developed (Ref year-1 report). These degenerate primers need further validation before universally accepted.

T01.05 - Estimation of incidence in tomato crops

Two methodologies were established to define the begomoviruses incidence. The first one: when only one virus occurred, is based on estimation by counting symptomatic plants; the second one, when more than one virus occurred is based on estimation of the relative incidence of each virus with molecular hybridization. A specific protocol was defined as technical information facsheet.

T01.06 - Identification of Weeds carrying the target begomovirus

A list of known begomovirus host species (weed and cultivated) was compiled based on the literature for each begomovirus. These and species found around and in tomato crops were tested for the presence of tomato begomoviruses. However, transmission tests to confirm the identified begomovirus hosts as potential virus sources to tomato crops were not carried out.

The survey identified a number of non-tomato infecting begomoviruses in weeds. eg. In Cuba: Jatropha mosaic virus in *Jatropha gossipyfolia*, Macroptylium mosaic virus in *Pseudelephytopus spicatus*. In Guadeloupe: Jatropha mosaic virus was detected in *Jatropha gossipyfolia*. Other distantly related

begomoviruses to known begomoviruses were detected in *Rhyncosia*, *Euphorbia*, *Merrimia*, *Corchorus*, *Sida* and *Malva* species. In Trinidad and Tobago: begomoviruses closely related to Calopogonium mosaic virus, Rhycosia mosaic virus and Sida golden mosaic virus were detected in *Rhycosia, Calopogonium* and *Sida* species. Specific virus host identifications were search using virus specific tools.

➔ **TYLCV**

In Cuba, a list of TYLCV hosts was published by Gonzalez (1995). TYLCV was detected for the first time in beans, pepper, squash and Euphorbia during the project period. In Dominican Republic cince it was extensively dealt with in another study by Salati et al (2002) Phytopathology 92 (5): 487-496, this was not pursued in the present study. In Guadeloupe and Martinique, of the species tested TYLCV was found only in bean. It was not detected in any of the listed TYLCV host species.

➔ **PYMV**

PYMV was only detected in pepper in Guadeloupe. None of other reported species were infected with PYMV.

➔ **PYMTV**

An exhaustive list of weed and host species were surveyed for the first time. PYMTV was only detected in pepper and at very low frequencies in Trinidad and Tobago.

T01.07 - Transmission biotest by B. tabaci from weeds to tomato and from tomato to weeds

Transmission biotests were not relevant since none of the weeds was found to harbour any of the begomoviruses infecting tomato production.

T01.08 - Determination of transmission rate of PYMV and TYLCV by B. tabaci Guadeloupe.

Tomato to tomato transmission tests were carried out on TYLCV and PYMV using 3, 10 and 25 adults. TYLCV was more efficiently transmitted to tomato than PYMV (Transmission efficiency was 60% for PYMV and 100% for TYLCV using 25 adults per plant). The transmission rates increased according to the number of females used for the transmission. This was not carried out for PYMTV.

T01.09 - Meeting 1 and training session in Trinidad

The first meeting and the laboratory session were held in Trinidad and Tobago, during the week of 13-17th October. Partner 4 organized it at the University of the West Indies in the campus of St Augustine. All participants were attending the meeting except the Cuban partners, Partner 6 and 7, due to political reasons. During the meeting, the work done in each WP was discussed and future work was defined. As the last time, discussion and interactions were intensive and performing particularly concerning the WPA and B. The WP C was discussed according data reported in the 18 month report and information given by partner 2, 4 and 1 who were involved in this WP and/or who recently visited Cuba. A report was done by the leaders (see annexes).

The laboratory session, which first aimed to training several partners to perform diagnosis of begomoviruses, was reorganized two months before due to the ability of the partners to do it themselves. The new aims were (1) to compare several protocols to extract plant DNA to define the best adapted to virus DNA identification and (2) to tests a set of primers for general diagnosis of begomoviruses. This laboratory session was held in the very well equipped Dr. P. Umaharan's laboratory and organized by his team, which is very relevant and professional. It was successful and a great moment. All partners sharing expertise and "how to do", were very enjoyed by this session.

WP B - Epidemiology and modelling to investigate the appropriate mix of disease control tools at both crop and ecosystem level

T02.01 - Workshop Wo1 on relationship between agrosystems and epidemics, development of a model.

The first workshop, held on in Guadeloupe, was also dedicated to consolidate knowledge on the factors influencing begomoviruses epidemics. So invited speakers and extension workers had participated and had contributed to inform Partner P2 (in charge of the development of a model) on local situations. Due to contrasted situations existing in the Caribbean islands, a comprehensive list of variables, which may be of importance in epidemic, was identified. Partner P2 provided a "maximal model" using General Linear Modelling approaches as the basis for the analysis of the data that will be collected during surveys. They produced with Partner P3 a draft survey design in order to establish why begomovirus diseases are more prevalent or severe in some places than others. This survey protocol was available on the BETOCARIB web site from the part restricted to the partners only.

T02.02 - Target survey to validate the agro systems model

The main objective of this task was to establish which of the variables in the "maximal model" have an effect on the response variables in order to determine a minimum adequate model. This will contain a smaller set of terms, all of which are significant. This new one defined as "agrosystem model" may differ among survey locations (two places in Guadeloupe and Three in Cuba). Establishing similarities and differences in the agro systems model for different locations are central to the aims of the work package.
Firstly the survey protocol established during the W01 was modified due to preliminary data collected through a series of pilot tests in Guadeloupe. These data were transcribed to Excel spreadsheets for subsequent analysis and **general linear models** were developed to establish which of the explanatory variables had significant effects on the response variables, disease incidence, severity and whitefly number. Of interest were not only relationships between explanatory and response variables, but also those between response variables themselves.
Firstly, the statistical analysis of correlation between individual variables remains difficult to assess because the variables are not independent and because some correlation can be significant merely by chance. Nevertheless, several relationships can be noted:

- Management practices and physical protection of the nursery were correlated suggesting that if the crop is looked after better, it is likely that this will include protection of the crop with plastic or glass houses.
- More surprisingly, physical protection of the nursery and previous hosts were also correlated. 'Previous hosts' refers to the presence of virus hosts in the vicinity, prior to, and at the time of planting. For whatever reason, it seems that physical protection of the nursery was more likely if previous host were abundant.
- Finally, whitefly hosts and virus sources were also correlated. This relationship may be linked to cropping intensity/diversity, with more diverse cropping systems being more likely to score highly for both whitely hosts and virus sources. The two variables might also be linked if tomato was the main crop grown, as the abundance of tomato would then be related directly to the abundance of both whitefly hosts and virus sources.

Secondly, the general linear model was fitted with these variables in order to keep only the set of all significant ones. The most important finding was that good 'management practices' were associated with both lower virus disease incidence and lower severity and correlated with 'physical protection' too. Management practices, however, made a consistently greater contribution to the model than did physical protection, so the former was retained in the model.The 'altitude' made a larger contribution to the model than did 'whitefly abundance' but the two variables were highly correlated. As expected, the age at which plants were scored and the date on which they were scored (i.e. the time in the season) both made very important contributions to the model. No effect was detected of tomato variety, insecticide use in the field or any of the other variables measured during the survey.
Statistical models based on the survey data have helped to establish what features of the ecosystems in Guadeloupe are associated with begomoviruses. These statistical models constitute the '**ecosystem models'** referred to in the project document. Separate models were developed to predict begomovirus disease incidence, severity and whitefly abundance. Models were developed for the two Islands of Guadeloupe, Basse Terre and Grand Terre. Were common patterns emerged; these might point to general predictors of begomovirus problems which might apply over a variety of locations.
The fit of these models to the disease incidence and severity observed in the surveys were good, especially for Basse Terre. The model predicting disease incidence in Basse Terre has an r-value of 0.93 indicating that the model explained more than 86% (r^2) of the variation in the incidence data.
Similar epidemiological surveys were also carried out in Cuba and Trinidad. The data entry was agreed, based on the spreadsheet used for the Guadeloupe survey but it was decided to modify the survey slightly in order to make it more meaningful for Cuban and Trinidad conditions. The results of the epidemiological surveys from Cuba and from Trinidad were analysed. For Cuba, two datasets were analysed separately based on data collected by IHLD and CENSA, respectively. Results were shown initially in the form of a series of contingency tables. In each case, these showed several significant associations between variables measured and disease incidence and/or severity. These significant variables tended to be linked and the linkages were examined further using principal components analysis.

T02.03 - Epidemic development and dynamics of B. tabaci in 3 representative sites of the Caribbean islands

Experiments were carried out mainly in Guadeloupe, in three different areas corresponding to different climatic and agro-environmental conditions. Studies were conducted regarding survey protocols established in the T02.01 and T02.02. The *B. tabaci* populations were monitored using a trapping system constituted by a tall mast, 6m high, equipped with yellow PVC cylinder at different altitudes (see annexes).

The climatic data were recorded using automatic data logger (temperature, relative humidity, rainfall, wind speed and direction.
Various climatic variables and natural enemy abundance were significant predictors of *Bemisia* populations. Both the temperature and saturation deficit have a positive effect on *Bemisia* populations, and the presence of parasitoids and occurrence of rainfall have a depressive effect. This indicates the adaptation of this species to hot and dry climates. The climatic parameters alone explain 53% of the observed variation.
Under certain conditions, local disease gradients from infected fields are relatively unimportant predictors of disease risk – more important may be the general level of background inoculum.
Even the climatic parameters may have a direct effect on the vector population (e.g. temperature or rain), other parameters such as cultural practice may be involved. For example, the presence of infected crops in the vicinity of the experimental plots, or the use of insecticides, which have a negative effect on the vector natural enemy populations. The inoculum pressure in different agro-ecological situations needs to be identifed.
There was a strong effect of the barriers on disease progress at least in the low-pressure of bemisia. Physical barriers were not efficient during periods of high primary inoculum, but may be combined with crop-free periods. This may reflect the fact that the whiteflies are concentrated, and/or developing inside the barrier plots.

T02.04 – Identification of biotic and abiotic (climate) factors prevalent in epidemics

In Guadeloupe the results show that three variables: good general management practices, physical protection in field and physical protection in nursery represent a package of measures which together were associated with ***low disease incidence***.
Although chemical protection had some association with lower incidence, its effect was quite weak. This may be partly due to the fact that chemical use appeared to be used as an alternative to the package of measures, which was strongly linked to low disease incidence. The result suggests a need to clarify the impact of whitefly insecticides on Begomovirus disease incidence under controlled conditions.
In Cuba ***high geminivirus disease incidence*** was associated with a combination of a greater abundance of previous hosts, greater host continuity and higher numbers of whiteflies, the growing of susceptible varieties, and a greater distance to the edge of the plot. ***Low disease incidence*** was associated with better management practices, later observation dates, location (more westerly sites), higher altitude, with chemical and physical protection in the field and the nursery, sheltered cultivation and good management practices. Findings from the Trinidad survey were not as conclusive as those from the Cuba survey and further discussion is needed to clarify the precise nature of some of the variables. However, it was apparent that location affected the time it took for begomovirus disease saturation to be reached. The presence of non-hosts was linked to location and, by association, with the longer time to reach disease saturation.
The survey results from Guadeloupe and Cuba were then synthesised and a table of common significant associations between disease incidence and the recorded variables was drawn up. This was used as the basis for the identification of candidate components for the IPM packages to be tested under WP C. Particular attention was paid to those variables that were negatively associated with disease incidence as these had the potential to be manipulated in disease management practices. Three variables in the ecosystem model in particular: chemical protection, physical protection and good management practices were associated with low disease incidence in **both** the Guadeloupe and the Cuba situations. This refers, respectively, to the use of imidacloprid to control whiteflies, various forms of crop screening using polyhouses, etc, and management practices such as weeding and irrigation. These aspects were indicated to be explored further in trials under WP C.
A complete analysis was realized comparing the results of all of the surveys (Cuba, Trinidad and Guadeloupe). A publication is currently being prepared which integrates the findings of the epidemiological surveys carried out in Cuba and Guadeloupe. The survey protocols differed slightly between locations, certain variables being appropriate in one location but not in the other. Statistical analysis of the two data sets has therefore been kept separate. Because the logistic regression approach used with the Cuba data proved productive and informative, for consistency, it is intended to redo the Guadeloupe analysis along the same lines and then combine the presentation of results in a single paper. The paper will make an important contribution in advancing epidemiological knowledge of begomoviruses, drawing on extensive data sets from two different locations.

T02.05 – Development of a crop model of epidemics

The 'Crop Model' is intended to simulate disease processes within a crop or a series of crops within a locality. Unlike the 'Ecosystem model' which examined statistical relationships between variables within

the ecosystem, the crop model is mechanistic in the sense that it describes disease processes using parameters that have clear biological meaning. This has the advantage that the model can be used to investigate the likely impact of management interventions. For example, in plots protected by cloth barriers, immigration of whiteflies is reduced. Immigration rate is one of the model parameters and so the relationship between immigration rate and disease progress in the crop can be explored using the model. A form of the model has been developed and verified which simulates disease progress over the period of a single crop.
From these experiments we have three pairs of disease progress curves together with corresponding data on the relative abundance of whiteflies in each member of the pair, (a) disease incidence on tomatoes under low inoculum conditions, with and without barriers and *with a yellow insecticide strip* (b) disease incidence on tomatoes under high inoculum conditions, with and without barriers (c) separate curves from the plots without barriers on TYLCV and PYMV based on virus specific detection in a sample of plants.
A draft publication was prepared, entitled: **Partially-protected cultivation to reduce the invasion of tomato crops by the virus vector *Bemisia tabaci*: modelling the impact on virus disease epidemiology.**
Abstract: In two experiments carried out in Guadeloupe barriers were used to reduce the entry of the virus vector, Bemisia tabaci, to tomato plots. The barriers erected around the crop were of insect-proof cloth fences 1.5m in height, in one case with an insecticide-treated, insect-attracting strip facing inwards. The two viruses present, tomato yellow leaf curl and potato yellow mottle, had similar disease progress curves and a general mathematical model of epidemic development was fitted to the symptom data from the treated and control (unprotected) tomato plots. Parameter estimates so obtained indicated that the barriers reduced vector immigration by approximately 12-fold but that B. tabaci retention within the plots was also increased slightly despite the mortality caused by the insecticide-treated strips. Disease establishment was delayed by approximately two weeks. Results from the other experiment involving barriers deployed without insecticide-treated strips could be explained by a large increase in B. tabaci retention within the barriers resulting in more rapid virus disease progress than in controls. Simple theory indicates that partial insect barriers can be worse than none because sufficient whiteflies can enter to establish a population and at the same time large numbers are retained in the barrier plot so increasing population growth.

WP C - Evaluation of IPM packages based on vrop management and resistant varieties

T03.01 - Screening varieties for resistance to the identified begomoviruses

Two methodologies were investigated in order to test the genotype resistance level to the begomoviruses: transmission by whiteflies and by grafting. A standardization of these two methodologies are made. Grafting inoculation test revealed its major celerity on TYLCV-Is transmission in tomato in comparison with whitefly inoculation method but when trying to evaluate genotype resistance under Cuban tropical conditions both tests, as described below, were efficient.
Six trials were carried out during this second year by Partner 1 (CIRAD-Martinique), Partner 4 (UWI-Trinidad & Tobago) and Partner 7 (IIHLD-Cuba), which involved 235 cultivars, for their resistance to the different begomoviruses reported in these countries (TYLCV-Cu, PYMV-Gu and PYMTV) and for other characteristic agricultural traits in different seasons of the year (dry and wet seasons).
Overall the studies made showed that:
1. It is possible to identify cultivars with broad resistance to a number of begomoviruses reported in the Caribbean Basin.
→ 'Vyta' (IIHLD-Cuba) and 'HA 3048' (Hazera Genetics-Israel) performed well under a high natural incidence of PYMV and TYLCV in the Caribbean North of Martinique where no plant health treatment applied.
→ 'HA 3018' (Hazera Genetics-Israel), 'Cuba 7', 'Cuba 24', 'Cuba 32', 'Cuba 42' and 'Cuba 57' (IIHLD-Cuba) showed promise to PYMTV in Trinidad & Tobago without chemical protection against whiteflies.
→ 'Cuba 7', 'Cuba 32', 'Cuba 39', 'Cuba 41' (former Cuba 42), 'Cuba 48', 'Cuba 57 ' (determinate growth habit) and 'Cuba 24', 'Cuba 60' (indeterminate growth habit) were asymptomatic under a high natural infection of TYLCV in Cuba without any protection. Besides, 'Vyta' is a cultivar well expanded in Cuba because of its resistance to TYLCV.

2. It is possible to select cultivars that provide general adaptability and market acceptability throughout the Region by testing a fairly broad spectrum of cultivars.
→ 'Vyta' and 'HA 3048' arose from the batch in Martinique also because their marketable yields (800 and 650 g/plant, respectively).
→ 'HA 3018', 'Cuba 7', 'Cuba 24', Cuba 32', 'Cuba 42' and 'Cuba 57' highlighted in Trinidad & Tobago also because of their productivity, ranging 50 to 82 t/ha.

➔ 'Cuba 7', 'Cuba 32', 'Cuba 39', 'Cuba 41' (former Cuba 42), 'Cuba 48', 'Cuba 57 ', 'Cuba 24' and 'Cuba 60' yielded 5 to 8 kg/plant in Cuba.
➔ 'Cuba 24' (2.5-12.5 mm) and 'Cuba 42' (5-10 mm) also highlighted in Trinidad & Tobago because of their firmness to a penetrometer (the lower limit refers to ripening fruits and the latter to the fully ripe fruits).

3. In this case the susceptible commercial cultivar 'Kada' (3-10 mm) is the standard based in the local requirements needed to acceptability.
Some cultivars seem to perform well in both, dry and wet seasons, while other seem to possess specific adaptability to particular conditions, suggesting a strong genotype x environment effect.
➔ 'HA 3018' performed well during the dry and wet seasons (50-70 t/ha, respectively) in Trinidad & Tobago.
➔ 'Cuba 7', 'Cuba 32', 'Cuba 39', 'Cuba 41' (former Cuba 42), 'Cuba 48', 'Cuba 57 ', 'Cuba 24' and 'Cuba 60' showed high yields ranging from 5 to 8 kg/plant in both seasons in Cuba.
➔ 'Cuba 42' highlighted in the dry season (50 t/ha) while 'Cuba 57' highlighted in the wet one (82 t/ha) in Trinidad & Tobago.

4. Begomovirus resistant cultivars should form the corner stone of any integrated pest management strategy developed in the fragile ecosystems found in the Antilles.
➔ The susceptible commercial cultivars used in the islands such as: 'Heat Master', 'Kada', 'Gempride', 'Gempear' and 'Gempack' can achieve 100% infection, even under low disease pressure, showing lower yields (below 30 t/ha).

T03.02 - Screening varieties to adaptation to Caribbean constraints

Several cultural practices including grafting may be adopted in order to improve the output of begomovirus resistant cultivars in areas of the Caribbean Basin where bacterial wilt (*Ralstonia solanacearum*) is also a strong constraint for tomato plants.
"Grafting Vyta" tomato plants were compared with "non grafting Vyta" tomato plants. Results of trials have confirmed the tolerance of Vyta to PYMV and TYLCV, and show the necessity to graft Vyta on a bacterial wilt resistant rootstock to have a production.

T03.03 - Workshop Wo2 and designing of IPM packages.

The second meeting was held in Cuba, during the week of 29th November to 3rd December 2004. Partner 6 and 7 efficiently organized it at the Plaza Hotel in Havana including a field trip at the end of the meeting and a visit of Cuban research institutes. During the meeting, the work done in each WP was discussed and future work was defined. As the last time, discussion and interactions were intensive and performing particularly concerning the WPC using a mind mapping approache in order to finalize IPM actions. Finally two IPM plots were defined, for Cuban and French West Indies conditions that will be compared with traditional method plots by Partners 1, 5 and 7(see annexes).The characteristics of the trials and the evaluations needed were also defined at the workshop.

T03.04 – IPM packages evaluation.

The three initial IPM plots were finally reduced to two during the Cuban meeting in order to avoid duplication and restricted to contrasted situations in terms of agro ecological conditions. These conclusions were established during the Cuban meeting on the bases of comparisons of several agro situations where similarities were observed between Cuba and Republican Dominican. These IPM plots were set up in Martinique and Cuba.
The final objective is to reduce begomovirus losses in tomato crop. Different factors were combined with regards to environmental conditions of producing areas:

- Use of a begomovirus resistant variety ('Vyta') versus a local commercial variety ("Amalia" in Cuba and "Heatmaster" in Martinica) susceptible to TYLCV;
- Use of insectproof isolated nursery to plantlet production versus a classic nursery (bare root, non isolated) in Cuba nad Martinica;
- Use of biological products (*Verticillium lecannii*) versus chemical products (Imidacloprid) to *Bemisia tabaci* control in Cuba;
- Use of grafting process to evaluate the adaptation to Caribean conditions particularly the tolerance to *Ralstonia solanacearum* in Martinica.

"Traditional practices + susceptible variety" lets a high viral severity which had a negative influence on tomato productivity "traditional practices + resistant variety" and "IPM practices with a resistant variety"

sygnificantly contributed to avoid damages. These results indicate that using a resistant variety in a correct IPM package bring a solution to grow tomatoes under TYLCV infected areas.

Problem encountered

Technical problems encountered in Workpackage A

1. Tomato-to-weed and weed-to-tomato transmissions
   - It was difficult to maintain infected weeds in greenhouse condition
   - It was difficult to maintain whiteflies on weeds and pepper for purposes of transmission tests.
2. PYMTV tomato-to-tomato transmission tests
   - Were not carried out due to insufficient whitefly rearing conditions in Trinidad. When conditions were established the purchase of cages was delayed due to insufficient funds.
3. Validation of primers
   - An adequate collection of begomoviruses was not available in any of the laboratories to validate the newly developed primers.
4. Difficulty to send diseased tomato samples from Dominican Republic to Cuba
   - Complicated administrative formalities hindered the timely approvals to send samples in a timely fashion.

Technical problems encountered in Workpakage C:
Martinique

1. Inconsistent results of grafting: problems of skill (beginning of the project), great effect of the high temperature on the decrease of grafting success rate.
2. Climatic problems in insectproof nursery – need for work on means to decrease inner temperature (mist trials).
3. Low virus disease pressure in the experimental plot of Le Carbet (particularly in 2004 and 2005): not enough for relevant varietal screening in the last two years
4. Availability of experimental plot in strongly affected area: implies to work in a grower's farm (possible follow-up constraints)
5. Interaction with bacterial wilt pressure: difficult to separate effects of viruses and BW on the overall yield depletion.

During the first year Partner P6 (Dominican Republic) and Partner P5 (Cuba) got some difficulties to interact. Partner P1 has played a co-ordinator role by emails and she has given them the possibility to exchange samples through the Guadeloupe station.The major problem encountered the second year concerned the impossibility for the Cuban partners to attempt the First Meeting due to very bad relationships between France and Cuba for the 6 last months in 2003. In order to release the situation, Dr P. Umaharan invited himself the Cuban people before the meeting, and during the first days of the meeting we tried to contact Cuba via the Embassy in order to explain the situation and find a solution. BETOCARIB is a typical regional project for the development of the Caribbean island and the absence of Cuban people highly panelized the discussions on WPC but also WPA and B. These different attempts were unsuccessful.
The major problem encountered the third year concerned difficulties of several partners to carry on research planned mainly due to financial problems. Actually, important and long discussions between the commission and the Coordinator regarding conformity of administrative papers blocked the realising of funds in time. This explains the non participation of the Dominican Republic partner to attempt the Second Meeting for financial reasons. The partners P04 and P05's absence highly panelised the discussions on WPC and a final solution was identified through intensive exchanges on two mind mapping IPM strategies provided during the meeting.
The major problem encountered the last year concerned difficulties of several partners to carry on research planned mainly due to financial problems and the project has been six months extended. IPM plots were only carried out by Partner 1 (Martinique) and 7(Cuba). Partners 4 (Trinidad and Tobago) and 5 (Dominican Republic) have not attended the second Meeting in Cuba because funds were not available. The solution identified during the meeting to overcome this problem was to intensify exchanges on the bases of the two mind mapping IPM strategies provided during the meeting. Unfortunately, no interactions occurred during this period resulting in the non effective participation of these partners on IPM plots.
The last meeting will also be postponed since the project has been extended.

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Conclusions

This project was the first opportunity for scientist from six countries to collaborate on the management of begomoviruses for sustainable production of tomato, a major problem in the Caribbean islands. Each time discussions and interactions were intensive and performing. Complementarities between project partners were very productive and strengthened scientific endeavour for all. New knowledge of begomovirus disease epidemiology in the Caribbean was established and quantifyed both the risk factors for disease and contributions of different disease amelioration measures. Inter-island comparisons enabled generalities and differences in the conclusions to be determined. The findings of the project feed through directly as disease management advice which can be tailored to tomato growers in the different islands involved in the project as well as allowing extrapolation with a fair degree of confidence to other Caribbean locations. All of this contribute to the success story of BETOCARIB.
Several direct and indirect benefits arise from the project. They are listed in the table below.

Direct	Indirect
Critical small mass of scientists to bring together facilities to address problems for working team	Critical small mass of scientists to bring together facilities to address problems for others
Increase knowledge of whiteflies, begomovirus and epidemiology in the Caribbean	Update of the begomovirus statute in the Caribbean
Tools for broad detection of the virus and whiteflies	Establishment of seed production and distribution system
Quantify disease risk factors	Collaborative partnerships were strengthened
Resistant varieties	Human resources sharing which lead to a multidisciplinary team to address problems
Multiple resistant to begomovirus	Good opportunity to give technical support to the farmers
Sustainable production of tomato	Strengthen the value chain between researcher, extension officer and farmer

The first benefit is that the project helps to address problems which were not affordable by a critical small mass of scientist in each country. Regarding scientific benefits, the project contributed to update and to increase the knowledge on whiteflies and begomoviruses in the Caribbean zone. Several useful tools for a broad detection of both viruses and vectors are developed and are validated. These tools are essential to study epidemics in tomato crops. A predicted model is established given the possibility to quantify disease risk factors. Finally useful and simple IPM practices are identified to decrease the impact of begomovirus epidemics including the identification of resistant tomato varieties. These IPM practices could be easily applied at the farmer's level and will contribute to significantly decrease the use of chemical treatments.
Several indirect benefits were also identified. The evidence that the multidisciplinary of the competence of the partnerships from academic to applied research contributed to solve the problem and to identified propositions - IPM practices- to transfer.

Future work might usefully further exploit the modelling tools that have been developed. In particular the crop model could be used to quantify the underlying epidemiological effects of other management interventions and so provide a rational basis for further improvements in practice
Following the final analyse of our results, we identified either several novel or unexploited scientific fields during the project that need to be investigated and can be proposed as future actions to progress.

Unexploited Scientific Subjects – Future Actions

- Research on durable (multiple-component) resistance against begomovirus in the Caribbean region
- Combine bacterial wilt resistance with begomovirus resistance
- Effect of the competition between TYLCV and bipartite begomovirus
- The threat of the evolution of begomoviruses on the viral situation in the region
- Risk of emergence of resistance breaking strains
- Risk of weed begomoviruses to become virulent in tomato
- Sustainability of virus control
    - durability of resistance
    - ensure stable resistance by good use of resistance varieties.

MANAGEMENT REPORT

Organisation of the collaboration

The organisation of the collaboration is based on duple time. The first one concerns official steps of collaboration based on official meetings such as annual meetings, workshops and one training session. The second one concerns a regular informal interaction to guaranty that each partner works to provide expected results in time. Then, a leader was identified by work package during the first meeting and the role and what it was expected were precise at this moment. Each leader was the scientific referent of the work package and systematically responsible of its management to the scientific co-ordinator. He played a major role in the beginning and the progress of his own WP. He interacted with the partners involved in his WP every time when it was necessary to set up protocols, to share out tasks, to ask about planning and results and to establish contact among different participants when it was difficult to do. The main interactions occur through an email exchange, which is smoothly going. The scientific co-ordinator interacted regularly with each leader in order to know how the work package progressed (facility or problem....). In some cases, this resulted in the identification of the needed of additional individual trainings to progress. At the beginning of the project, a BETOCARIB Web site has been created to formalise the interactions between consortium members and to keep us aware on the project progress. Due to these objectives, the Betocarib Web site has been divided into two parts: everybody can consult one and one restricted to members only and be devoted to keep official protocols, guidelines, facsheets, administrative documents etc... usefull to well manage the own partner's work. The web site can be accessed at the following address: htpp://betocarib.cirad.fr

Meetings

Three meetings, two workshops and a training session were planned and realized during the project.
The first one, the launching meeting, was held from 4th to 8th March 2002 in Guadeloupe. Partner P1 and P3 organized it at INRA Petit-Bourg station. All the seven partners were attending the whole session.Two invited scientists, Dr Judith Brown (Tucson University – Arizona) and Dr Douglas Maxwell (Wisconsin University) also joined this meeting. Their input were highly beneficial, especially regarding characterization of both vector "Bemisia tabaci" and virus by sharing their experiences on the begomoviruses surveys in vegetables in Central and Latin America, and in Africa. Their presence helped us to define the best-appropriated ways to adopt. The organization of the first meeting and Training session in Trinidad is in progress.
The first meeting and the laboratory session were held in Trinidad and Tobago, during the week of 13-17th October. Partner 4 organized it at the University of the West Indies in the campus of St Augustine. All participants were attending the meeting except the Cuban partners, Partner 6 and 7, due to political reasons. During the meeting, the work done in each WP was discussed and future work was defined. As the last time, discussion and interactions were intensive and performing particularly concerning the WPA and B. The WP C was discussed according data reported in the 18 month report and information given by partner 2, 4 and 1 who were involved in this WP and/or who recently visited Cuba
The laboratory session, which first aimed to training several partners to perform diagnosis of begomoviruses, was reorganized two months before due to the ability of the partners to do it themselves. The new aims were (1) to compare several protocols to extract plant DNA to define the best adapted to virus DNA identification and (2) to tests a set of primers for general diagnosis of begomoviruses. This laboratory session was held in the very well equipped Dr. P. Umaharan's laboratory and organized by his team, which is very relevant and professional. It was successful and a great moment. All partners sharing expertise and "how to do" were very enjoyed by this session.
The second meeting was held in Cuba, during the week of 29th November to 3rd December 2004. Partner 6 and 7 efficiently organized it at the Plaza Hotel in Havana including a field trip at the end of the meeting and a visit of Cuban research institutes. All participants were attending the meeting except the Dominican Republic and Trinidadian partners, Partner 5 and 4. Partner 5 has got financial problems due to expected funds from the European Commission related to the providing of the Second annual report. Partner 4 was delayed in Jamaica and he couldn't take his plane in time to join us in Cuba.
During the meeting, the work done in each WP was discussed and future work was defined. As the last time, discussion and interactions were intensive and performing particularly concerning the WPC. A report and planning work was provided by the leaders and send each other to stop the final actions.
The last meeting will be postponed since the project has been six months extended. Partner 5 will organize it at ISA, Santiago in Republic Dominican. During the meeting, the last results concerning IPM practices WPC and predicting model will be discussed in order to finalize concrete actions permitting to decrease the begomovirus incidence in tomato production in Caribbean zone. All possibilities to

disseminate scientific information at a country level will be explored too with each partner. Final report including the major results will be discussed in order to produce an exploiting data.
Each time discussions and interactions were intensive and performing. Techniques, procedures, protocols and details to perform each task were stated. Each Partner left the meeting every time with a clear idea of what has to be done exactly to reach maximal efficient.

Exchanges

During this first year visits between Partners were:

- Partner P2 arrived in Guadeloupe a couple of days before the Wo 1 to visit several productions areas with Partner P3 in order to define the most adapted methods to set up the trials of WP B.
- Partner P7visited Partner P1 (Martinique) three days before the Wo1 in Guadeloupe.
- Partner P1 (Martinique) visited the different systems for vegetable production in Cuba in April 2002 hosted by Partner P7.

During the second year several visits was also realised:

- Between Partner P2 and Partner P6 &P7 to set up the trials of the WP B like in Guadeloupe in March 2003, and Partner P6 and P5 to treat all samples collected.
- Partner P6 has submitted a demand for a scholarship to allow a member of the team to learn the techniques for the characterization of *Bemisia tabaci* in the Partner P1's laboratory in Montpellier. This is to supplies the difficulties to exchange material.

During the third year:

- Partner P4 invited himself Partner P6 who didn't reach the laboratory session organized at the same time than the first meeting.
- Partner P7 visited Partner P4 and discussed about the set up of trials for WPC.

During the fourth year, Partner P7 visited partner P1 in Montpellier during one month in order to treat herself bemisia samples collected in Cuba.
Then partners P2 visited three times Partner P3 in Guadeloupe and two times partner P6 and P7 to develop a predicted model and propose a final one adapted to the Caribbean constraints.
Partner P1 Martinique strongly interacted with Partner P7 to plan all plots and actions related to WPC.

INDIVIDUAL PARTNER REPORTS

INDIVIDUAL PARTNER P1 FINAL REPORT

PARTNER P1: Centre de Coopération internationale en Recherche Agronomique pour le développement CIRAD (France)

CIRAD GUADELOUPE - Cica Urbino

1. Objectives

Begomoviruses are the major viral constraint of tomato in the French Caribbean islands. CIRAD GUADELOUPE is implicated in sustainable development of tropical horticulture and one of its objectives is to have a better knowledge of the emerging diseases and to develop integrated pest management strategies. We have an experience on both virus characterisation and field epidemiological studies. The laboratory of virology of CIRAD – INRA based in Guadeloupe has all the facilities to conduct molecular analysis and biological characterisation of begomoviruses. Our objectives in the present project were to characterise the begomoviruses involved in tomato disease in Guadeloupe and Martinique, to determine their respective incidence, and the weeds which were the sources of these viruses. All these activities were included in the workpackage A of the project. I was responsible of this workpackage.

2. Activities

The WP A procedures were defined during the first meeting in Guadeloupe during the first workshop hold on at the beginning of the project in March 2002. The different presentations and the discussion among all partners allowed having a good overview of the ecological conditions in the tomato growing area in the different islands. During the meeting, we discussed and agreed about the procedures and the protocols necessary to realise the different tasks of work package A described in the final document (T01.01 to T01.08). A first report was written and sends to the different partners to start at the same level of information.

Begomovirus characterization

Since 1993 tomato plants were infected in Guadeloupe and Martinique with a bipartite begomovirus already identified as potato yellow mosaic virus (PYMV). New symptoms closed to those described for tomato yellow leaf curl begomovirus (TYLCV) were observed on tomato in field in Guadeloupe in 2001 and in Martinique in 2002: leaf reduction, leaf curl, yellowing of leaves. In a first approach of characterisation, samples were collected in different tomato production areas of Guadeloupe and Martinique in November 2002 and June 2003. They were tested with ELISA and PCR with degenerated primers for begomovirus detection; PCR products were then cloned and sequenced.

The diversity of sequence of each identified begomovirus was studied through restriction analysis on PCR amplified fragments.

Development of specific tools for diagnosis of the identified viruses

Different tools were needed for the detection of begomoviruses in plants. Specific tools have to be developed to detect and distinguish different begomoviruses in case of mixed infection in a country. We chose the molecular hybridisation technique which allows the treatment of many samples easily, and can be use as a routine technique. Probes were obtained with the labelling of PCR fragment corresponding to the intergenic region of each virus (PYMV and TYLCV). A generic probe able to detect different begomoviruses was also developed from the core coat protein fragment of both TYLCV and PYMV. Detection with chemiluminescent substrate (Amersham) was applied to increase the threshold sensitivity of the detection. The protocols were adapted to obtain a specific reaction for each virus. These probes were evaluated for efficiency.

A protocol for begomovirus detection in weeds was required. Indeed, some plants give sticky or phenolic extracts which do not enable a correct diagnosis of begomoviruses. We first looked for the best protocol to extract DNA from these plants and then we have tested different techniques for diagnosis (PCR, hybridisation).The training sessions at Trinidad and Tobago was focused on this point.

Estimation of incidence on tomato crops

Two surveys were realised in the tomato producing areas of Guadeloupe and Martinique. Eight to thirteen fields were prospected in December 2002 and in June 2003 in each island. Around 40 samples were collected per field. Specific diagnosis of begomovirus was achieved through molecular hybridisation.

Identification of weeds carrying the targeted begomoviruses
A list of weeds already known to be infected with begomoviruses (TYLCV and /or ToHMV in Cuba and Dominican Republic, PYMV in Trinidad and Tobago) was established from previous results. This list was completed with the different weeds species present in and around tomato fields in each island.
Samples from these species were collected in Guadeloupe during a first survey in 12 infected tomato fields (6 fields in Grande-Terre, 6 in Basse-Terre). 650 samples of weeds corresponding to 22 families and 50 species (including species which were already known to be hosts of TYLCV and PYMV in the Caribbean) were collected. ELISA tests (using TYLCV kit from Adgen) and molecular hybridisation with the generic probe were conducted on the sap and DNA extract. Positive samples were then blotted on membranes for the diagnostic of TYLCV and PYMV with specific probes.

3. Results achieved
The first conclusion of the characterisation work done in Guadeloupe and Martinique in 2002 concluded that tomato was infected with two begomoviruses: PYMV and TYLCV. TYLCV is considered resulting from a recent introduction and the way of introduction is yet unknown. The intergenic region (580 bp) and the complete coat protein gene (980 bp) of the TYLCV were sequenced for several samples from Guadeloupe and Martinique. These parts of the begomovirus genome are necessary for the identification of the strain according to ICTV (International Committee on Taxonomy of Viruses) and for publication. The four sequences showed at least 98% identity to each other and at least 98% identity with TYLCV isolates from Cuba and the Dominican Republic which belong to the TYLCV-Israel species. The nucleotide sequence of the TYLCV isolate from Guadeloupe was established for 2507 nucleotide out of a putative sequence of 2787 bp and this sequence is 97% identical to that of TYLCV isolates from Cuba or the Dominican Republic. Comparison of the sequences of the different ORF has confirmed this result.
Restriction analysis revealed that for almost all samples the same profile with that of the sequenced isolate of either TYLCV or PYMV was obtained. The few different ones which gave different profiles were sequenced and the results confirmed the identity of PYMV or TYLCV with minor differences in the restriction sites. It can be concluded that there is few or no diversity of the two begomoviruses in Guadeloupe and Martinique.

Development of specific tools for diagnosis of the identified viruses
As defined in the meeting of Guadeloupe, the diagnosis will be realised by hybridisation with PCR labelled probe. This technique enables to treat easily a lot of samples. The AlkPhos system provided by Amersham was used. Specific probe were defined for each virus and tested against different begomoviruses. The intergenic region was chosen because of the variability of its sequence among the begomovirus group A 450 bp and a 600 bp fragment covering this region were chosen for each begomovirus. The protocol was improved by concentrating the plant DNA extract by an isopropanol precipitation. The probes were tested for sensibility and specificity. We found that the detection was possible for extract dilution until 10-4 and for PCR fragment until 10-8. Specificity was established for PYMV probe. This technique allows the treatment of many samples for the estimation of incidence or the epidemiological experiments.
The protocol which was chosen for the extraction of DNA from weeds was Dellaporta's protocol. PCR was then conducted on these DNA extracting samples using degenerated primers MP16-MP82. Kobayashi's extracting protocol which was proposed first was difficult to performe and the presence of PCR inhibitors in the DNA extraction impeded the diagnosis.

Incidence of begomoviruses in fields in Guadeloupe and Martinique
In December 2002, PYMV and TYLCV were well established in the principal tomato producing areas of Martinique (North Caraibe) though only PYMV occurred in the North Atlantic coast. Six month later, the situation was the same or worse in the North Caraibe area; TYLCV was detected for the first time in the North Atlantic coast. Begomoviruses were present in fields from the third week after plantation; the incidences of the two viruses were equivalent but in some fields, TYLCV was predominant. During both surveys we did not observe or detect begomoviruses in tomato in the South of Martinique. This situation was conforming to precedent report in Martinique. This can be explained by the fact that the climatic conditions in the South were not suitable for vegetable crop (very dry), so tomato crops were isolated one from another and also from the other tomato producing areas. Nurseries were located closed to the fields in the South and there were few no exchanges of tomato plants with the North.

In Guadeloupe, the situation was similar to that of Martinique: the two begomoviruses were present in the two principal tomato producing areas but fields which were isolated from theses areas (Abymes or INRA

station Petit Bourg for example) were scarcely infected with begomoviruses. Symptoms of TYLCV were predominant in all fields with incidence of 60 to 100% in the two regions. In case of mixed infections, symptoms were TYLCV typical symptoms.
In the two islands, the situation was not different between the beginning (December) and the end of the production season (June). The incidence of begomoviruses in tomato was high because tomato fields are present all over the year and they are very closedone to another in the intensive tomato producing areas.

Identification of weeds carrying the targeted begomoviruses
Begomoviruses were detected in many weeds species with either by ELISA or by the generic probe but neither PYMV nor TYLCV were detected in any species tested even those which were cited to be TYLCV or PYMV hosts in other region in the Caribbean. The sequences of begomoviruses detected in these weeds were closed to different already known begomoviruses as Jatropha mosaic virus, Euphorbia mosaic virus, Rhynchosia mosaic virus, etc. It seems that these two tomato begomoviruses were not infecting weeds in Guadeloupe.
TYLCV was detected in *Phaseolus vulgaris* (2 /10 plants) in Grande Terre, Guadeloupe and PYMV was detected in sweet pepper only when the field was closed to an infected tomato field. Vector transmission from tomato to sweet pepper was shown but the transmission from pepper to tomato was difficult since whiteflies did not survive well on pepper. Only few plants of bean and pepper are cultivated in Guadeloupe. Then, we think that the principal inoculum source must be tomato.

Transmission biotest by *B. tabaci* from weeds to tomato and from tomato to weeds
Since we did not find any weeds carrying PYMV and/or TYLCV, we did not have to make transmission tests from weeds to tomato

4. Problem encountered
None

5. Technology implementation plan
Not applicable

6. Publications and papers
Urbino C., J. Polston, C. Patte and M.L. Caruana. (2004) - Characterization and genetic diversity of Potato yellow mosaic virus from the Caribbean. Archives of Virology, 149(2): 417-424.
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Communication
C. Urbino, K. Tassius, A.L. Gérion, C. Marchal and H. Vignes (2003) - Incidence of begomovirus diseases in tomato crops in Guadeloupe and Martinique. - French meeting of Plant Virology 2-6th of February 2003, Aussois, France
C. Pavis, C. Urbino, M. Marquier, J. Agrapart, N. Robin, L. Rousseau, D. Lange, and N. Boissot (2003) - Epidémiologie des maladies à begomovirus en Guadeloupe. - French meeting of Plant Virology, 3-7th of February 2005, Aussois, France

Training
Benedicte Laborie (2003) - Identification of alternative hosts of TYLCV and PYMV among weed species in Guadeloupe - June to September 2003

CIRAD MARTINIQUE - Christian Langlais PRAM CIRAD

1. Objectives
WPC was supposed to define the adequate IPM strategies to control viruses based on the use of tolerant/resistant varieties, the cultural methods and the prediction of infections due to the agronomic epidemic model developed. In the case of Martinique, resistance to bacterial wilt and to the heat will also be necessary. The evaluation of the IPM strategies would consider varietals resistance to viruses and bacterial wilt even with grafting, the use of insect proof nursery, and to test packages of cultural techniques.

Varieties evaluation

Trials were set up in virus infected zone (Le Carbet) and in uninfected zone (le Lamentin). In both zones, bacterial wilt is present and more aggressive during the hot and rainy season.

Grafting
As we could not get any varieties combining both resistances to begomoviruse and to baterial wilt, we tested alternative solution by grafting begomoviruses resistant varieties onto bacterial wilt resistant rootstock.

Insect proof nursery
The objectives were
- to evaluate if the plants resulting from insect proof nursery (A) will be infected later than plants sown in infested zone;
- to evaluate if the output in field of the plants (A) will be less affected than that of the plants (B);
- to measure the impact of the climate under and outside insect proof nursery;
- to compare the growth of the tomato seedlings under insect proof nursery and under traditional nursery;
- to evaluate the effect of the quality of the nursery on the output ("delay to the infestation" due to the use of healthy seedlings).

Evaluation of an IPM package
IPM package were designed for Cuba and Martinique. In both cases, the aim is to significantly lower the impact of begomovirus on tomato crop.
In Martinique, the combination of different factors was evaluated:
Nursery in insect proof or classic conditions,
- Grafting of tomato crops onto bacterial wilt resistant rootstock or non grafted,
- Using of classical variety (ie Heatmaster with tolerance to Ralstonia solanacearum and nematods) or improved variety (ie Vyta with tolerance to TYLCV, but no tolerance to R. solanacearum).

Two trials have been set up, (i) one at the end of the dry season and (ii) another one during the rainy season in "le Carbet" which is located in an important zone of infestation by begomoviruses.
At the end of the dry season, populations of *Bemisia tabaci* could be high and the risk of infection is high too once the risk of bacterial wilt is low,
During rainy season, the risk of bacterial wilt could be high once the risk of begomovirus infection is low.

2. Activities
Varieties evaluation
A total of 4 trials have been conducted: 1 in 2002 (Le Lamentin), 1 in 2003 (Le Carbet) and 2 in 2004 (Le Carbet and Le Lamentin). All trials have been planted in soil. Plantation in Le Lamentin October 2002, and March 2003, in Le Carbet March 2003, and 2004 and June 2004.
Varieties were coming from Petoseed : Heatmaster (WITNESS), from Cuba : Vyta, Cuba 7, Cuba 12, Cuba 32, Cuba 42, Cuba 48, Cuba 57, from Hazera : Ha 8479, Ha 3012, Ha 3017, Ha 3018, Ha 3019, Ha 3022, Ha 3048, Ha 3052, Ha 3059, from INRA Caraïbo, Hawai7996, L180-1, L390, Roma, Sadik, Sumo

Grafting
Grafting has been tried in 2004 and 2005. The rootstocks are resistant to bacterial wilt (Caraïbo or Hawai 7996).
Several trials were made to evaluate the grafting technique proposed by AVRDC in order to avoid the risk of bacterial wilt contamination.
Stems of scion and rootstock must be the same diameter, 1.6–1.8 mm. The rootstock plant is cut above the cotyledons at a 30° angle. The scion stem should be cut at a 30° angle, slightly above the cotyledons or first true leaf. A 10-mm-long latex tube (2.0-mm-inner diameter and cut at a 30° angle) is slided over the scion stem. The cut angles of the tube and scion are parallel. The scion is pushed about halfway into the tube and slided over the rootstock seedling stem. Again, make sure that the cut angles of the tube and rootstock stem are parallel. The grafted seedlings are kept into the shaded chamber. Four to five days after grafting, begin the hardening process by peeling away the top layer of shade net material. The entire process takes 30 to 33 days from sowing.

Insect proof nursery
Insect proof nursery was evaluated in 2003 in Le Lamentin zone and in 2005 in Le Carbet zone. Each time we measured temperature under nursery and compare it with the outside one.

Evaluation of IPM package
The objects tested will be:
Dry Season Trial:
classical package: variety Heatmaster (tolerance to bacterial wilt) grown under classical nursery ;
improved package: variety Vyta (tolerant to begomovirus) grown under insect proof nursery, No grafting.

Rainy Season Trial:
Classical package: variety Heatmaster (tolerance to bacterial wilt but sensible to begomovirus), grown under classical nursery (without insect proof), duration of the nursery stage = 20 days ;
IPM with classical nursery: variety Vyta (tolerant to begomovirus) grown under classical nursery, grafted on Caraïbo (rootstock with tolerance to bacterial wilt) ;
IPM with insect proof nursery: variety Vyta (tolerant to begomovirus) grown under classical nursery, grafted on Caraïbo (rootstock with tolerance to bacterial wilt).

In order to check the level of infestation in *B. tabaci* and in R. solanacearum, a pre trial has been made on the same plot. This plot was cultivated in cucumber, so we added plants of sensible variety of tomato (Roma) in the field.

3. Results achieved
Varieties evaluation
Trial in non infected zone 2003
Varieties tested in soil less culture under shelter in Lamentin (TYLCV non infested zone):
The best marketable yields were obtained with Ha 3052, Ha 3022, Heatmaster, Ha 8479, Cuba 57 and Vyta. The bigger fruits were from Ha 3022, Heatmaster, Ha 3052, Ha 3017.

Trial in TYLCV infected zone 2003
The trial was conducted in soil culture in Le Carbet, a TYLCV infested zone.
The bacteria *Ralstonia solanacearum* leaded us to graft half of the plants for each variety on the variety Caraïbo which has a resistance to these bacteria. The varieties were from Hazera (HA 8479, HA 3017, HA 3022, HA 3048), from IIHLD (VYTA) and the witness from Petoseed (HEATMASTER).
The virus infection was evaluated by symptoms notation done every 15 days (Scale of notation: 0 = not of symptoms; 1 = light mosaic or deformation; 2 = mosaic or rolling up of the sheets without repercussion connects on fructification; 3 = deformation and strong mosaic; 4 = stunted plants, general crispation of the vegetative apparatus, general run-out of the flowers).
The appearance of the first symptoms was raised about fifteen days after the plantation. The various symptoms observed are: - rolling up of the sheets in the shape of spoon - yellowing and reduction of the size of the sheets – dwarf plants. The rate of contamination evolves very quickly. For the grafted seedlings, Heatmaster is the most quickly reached (scale 4 three weeks after plantation), HA3022 has got the lowest note (1.5 four weeks after plantation), the other varieties having intermediate behaviours. For the not grafted seedlings, Heatmaster and HA8479 are reached (note 3 four weeks after plantation), the other varieties not exceeding the note of symptoms "2".
An analysis carried out on one plant from each variety by the Service of the Protection of the Plants detected a geminivirus in all the samples except for HA 3048 for which *Phomopsis sp*; and *Xanthomonas campestris pv vesicatoria* were found.
The grafted seedlings give a harvest by plant more abundant than the not grafted seedlings.
For the grafted seedlings, the Vyta and HA 3048, give the best outputs (marketable fruit 800 and 650 g/plant) that it is in marketable harvest or total harvest. Heatmaster arrives in third position.
For the not grafted seedlings, HA 3048 leaves the batch with 600 g/plant of marketable fruits, the other varieties not exceeding 400 g/plant.
HA 3048 gives of the same fruits average weight as Heatmaster (160 g/fruit), whereas Vyta has smaller fruits (120 g/fruit).
The virus tolerance of VYTA and HA3048 can be combined with the grafting technique (against Ralstonia solanacearum) in order to produce tomato, waiting the availability of a variety having both tolerances.

Trial in non infected zone 2004
A variety trial has been conducted to evaluate the resistance to Ralstonia solanacearum from different varieties: Caraïbo, Heatmaster, Hawai7996, L180-1, L390, Roma, Sadik, Sumo and scion of Heatmaster and Roma grafted on Hawai7996 and Caraïbo. The trial has been planted in June in open field in Le Lamentin in ferrisol infected by Ralstonia solanacearum.
80% of the dead plants have been registered one week after the plantation.

The variety Cuba 48 have a high death rate and had also the lowest germination rate (45% compare to 74 to 98% for other varieties). Cuba 12 and Heatmaster have the lowest death rate (8 and 11%).

Trial in TYLCV infected zone 2004

Another varieties trial has been set up in June with varieties from Cuba (Cuba 7, Cuba 32, Cuba 48, determinate growth and Cuba 12, indeterminate growth) and from Petoseed (Heatmaster). This trial has been set up in Le Carbet which is a TYLCV infected zone, but the Bemisia infestation has been poor.

Virus symptoms

no symptoms were observed on the Cuban varieties, and only 6 plants out of 133 (4%) have symptoms on Heatmaster with only 2 plants reaching the note of 3. In fact, the Bemisia infestation was very low at the beginning of the trial and did not increased during the trial.

Harvest

variety	marketable yield kg/ha	unmarketable yield ka/ha	average weight of fruits in g
Heat Master	1.60 a	0.8	142 a
Cuba 32	1.54 a	0.98	80 b
Cuba 48	0.72 b	0.62	144 a
Cuba 12	0.55 b	0.47	110 b
Cuba 7	0.44 b	0.43	79 b
cv	57	40	16
etr	0.59	0.27	17
signification	0.04	0.05	0.002

Cuba 32 has equivalent yield to the one of Heatmaster.

The non commercial fruits are due to bird and rat damages.

Cuba 48 and Heatmaster have the same average commercial fruit weight (around 140 g/fruit). Cuba7 and Cuba 32 have a lower average fruit weight (110 to 80 g/fruit).

Fruits' quality

Firmness: between the two harvest dates, the firmness increased for all varieties but Heatmaster. Heatmaster has the lowest firmness, while Cuba 48 and Cuba 7 has the best.

Acidity: pH is decreasing along the cycle. Heatmaster, Cuba32 and Cuba12 are the more acid.

Duration of fruit storage: the % of rotten fruits is more important in the second week for all varieties. During the first week Cuba 32 and Cuba 12 have less rotten fruits than Heatmaster.

Conclusion

The Cuban varieties were not contaminated by TYLCV. Only 6 plants (4%) of Heatmaster show some symptoms, which is low and does not induce any yield reduction. The whiteflies infestation was very low; therefore it is impossible to conclude on resistance to TYLCV among the different varieties. Regarding the harvest under low pressure of TYLCV, Heatmaster and Cuba32 are the best, regarding the average size of the fruits Heatmaster and Cuba48 have the biggest fruits. Heatmaster has fruits with lower firmness and higher acidity.

Grafting

2004

Success with latex tube was highly variable between 0 and 64%, success with grips were between 48 to 71%.

Compared to the latex tube the grips to be grafted are:

- Easier to use,
- The grips fall from themselves and can be re-used another time.

2005

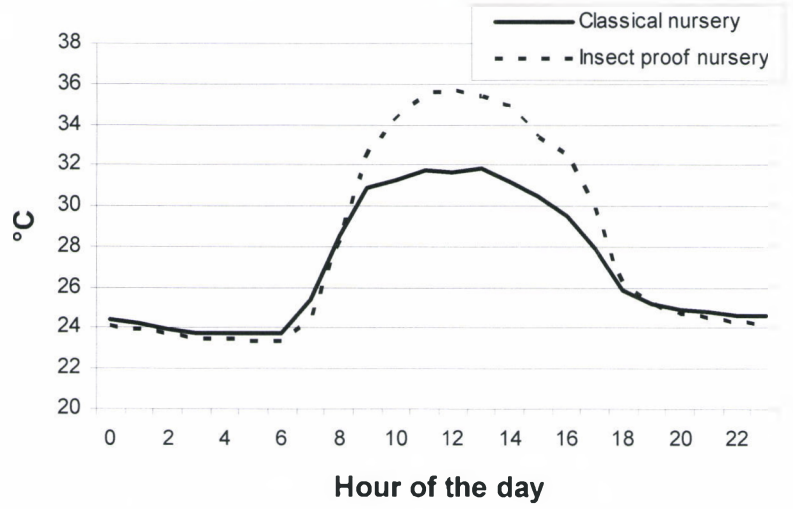
Grafting was delayed under insect proof nursery (20 days between sowing and grafting compared to 13 days under classic nursery): the plants did not emerge well and did not grew well. The rate of success of the grafting was low under insect proof nursery (less than 40%). The higher temperatures under the insect proof nursery may have caused these damages.

Insect proof nursery

2003

Climate under insect proof nursery
The daily average temperatures are 1.5 to 2°C higher under insect proof nursery than outside.
At the hottest moment of the day, it makes to on average 4°C moreover under the nursery insect proof that outside. However if the day is sunny this difference can go up to 8°C and the temperature then reaches the 40°C under the nursery.
Growth of the seedlings under insect proof
The seedlings under insect proof nursery are larger and heavier than those under traditional nursery. This faster growth is the consequence of the rise in temperature. A nursery under anti insect thus allows to reduce the duration necessary to obtaining the seedlings. However, this nursery not being completely tight (insect proof net), it will have to be checked that in rainy period there is no development of disease.
Effect of the quality of the seedlings (infested in nursery or not) on production
Evolution of the symptoms
Plots with healthy seedlings (resulting from Lamentin nursery) and planted in a healthy zone (Lamentin) do not present any symptom. Plots with seedlings raised in infested zone (resulting from Carbet nursery) and planted in an infested zone (Carbet) are very quickly infested. Plot with healthy seedlings (resulting from Lamentin nursery) and planted in an infested zone (Carbet) is intermediate: the plantation remains healthy during three weeks then the infestation starts and the curve of note of the symptoms is shifted one week compared to that of the seedlings raised in zone infested at the beginning of cycle then of two week in medium of cycle.
Production
The plants of Carbet having undergone a strong attack of acariose combined with the infestation by the begomovirus did not produce. For the plot in Lamentin, the first sowing suffered more at the time of the recovery, the seedlings were too old ; on the second sowing one observes a production of 1.1 kg/plant for 6.5 fruits.
Conclusion
The insect proof nursery made it possible to produce seedlings more quickly than a traditional nursery because of a higher temperature. It will be necessary however to adapt work under insect proof nursery since in sunny day the temperatures can go up to 40°C. It will be necessary to check that in rainy period once there is not a development of the disease since this nursery is not completely tight for rain (insect proof).
The technique "healthy seedlings in infested zone" makes it possible to delay the contamination from eight to fifteen days compared to seedlings raised in infested zone, but this delay with the infestation is not sufficient to obtain a correct production in a much infested zone

2005
During the trial on IPM packages in Le Carbet, temperatures were recorded under the classical nursery and under the insect proof nursery.



At 12 am the temperature under the insect proof nursery is 4°C higher than the one under classic nursery, reaching 36°C in average over the period. In order to decrease the temperature, we put a shade net on 9/11/2006, but that was not enough.

IPM Package 2005

Pre trial: infestation in B tabaci, R. solanacearum, begomovirus and nematods
Material and methods
The plots C3 and C4 were cultivated in cucumber, we added some tomato plants: one line of Heatmaster on both sides of the plot and plants of Roma in the middle of the plot.
Plantation was done on 15 and 16 february 2006.

B. tabaci population:
Yellow sticky board is disposed in the plot to trap B. tabaci. Disposition and high of the traps (C. Pavis INRA) : a PVC tube of Ø8cm and 30cm length is pushed 20 cm into the soil, 10 cm are up the soil. On this tube we dispose the yellow sticky traps (10cm x 10 cm). Six traps have been put in the plot on the 22/02/05 and are changed every week. Sticked *B. tabaci* are counted.

Viral symptoms and wilting due to bacteria
Every week, number of plant having virus symptom (TYLCV or PYMV) and number of plants being wilted are counted. The last one are collected with some soil and sent to the lab. There classical bacteria isolation, and multiplex PCR is been proceeded.

Results
Bacterial wilt and nematods on Roma
18 plants have been taken from the field, and ten of those had a positive water test. Six were also positive on bacterial test and all were belonging to the phylotype 1. Rate of infestation in Ralstonia solanacearum is low (4 % of the plants).
Only one plant on the 18 was having some nematods galls.

Counting *B. tabaci* on sticky traps
The number of *B. tabaci* is increasing from 25 adults (8/03/05) on a sticky trap of 10xup t10cm up to 300 (57 days after plantation).

Viral symptoms
On 18 april 2005, thirty apex samples were taken at random. Begomovirus were detected on all samples (test ELISA).

Evaluation of a IPM package during the dry season

Materials and methods
Objects
Classical package: variety Heatmaster (tolerance to bacterial wilt) grown under classical nursery;
Improved package: variety Vyta (tolerant to begomovirus) grown under insect proof nursery, no grafting.

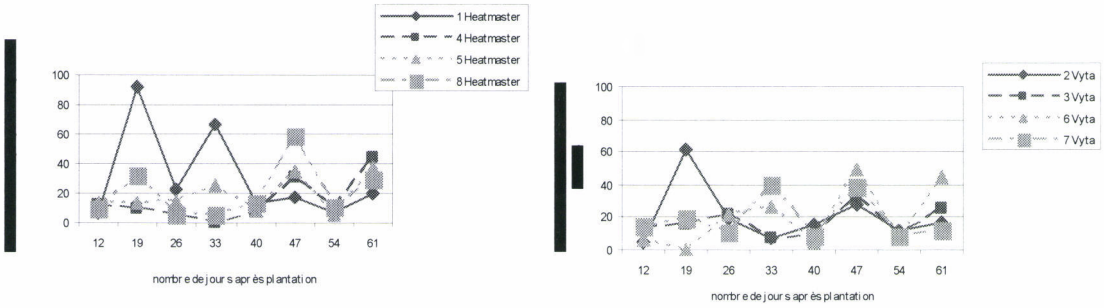
Lay Out
Plots C3 and C4 (22m x 18m), 20 lines, space between lines 1.2m, density 2 plants/m2
Elementary plot: 5 lines of 20 plants, 100 plants in total. 4 blocs.
Sowing 09 may, Plantation 27 may, 1st harvest 28 july, last harvest 5 august No pesticid was applied on the plot.

Measurement and observations
B. tabaci population
After plantation, 8 yellow sticky traps are set up in the plot, in the middle of each elementary plot and changed every week.

Virus symptoms
After nursery: on 30% of the plants are keept six days more in the nursery before to be tested in molecular hybridization, all other plants are planted.
After plantation:.every week, the plants with symptoms on 40 plants are counted. Scale is the one of Scoot and Schuster : O nothing, 1 begining, 2 average, 3 severe.
Lab analysis: method molecular hybridization Amersham.

Other counting
On each elementary plot, flowers are counted.

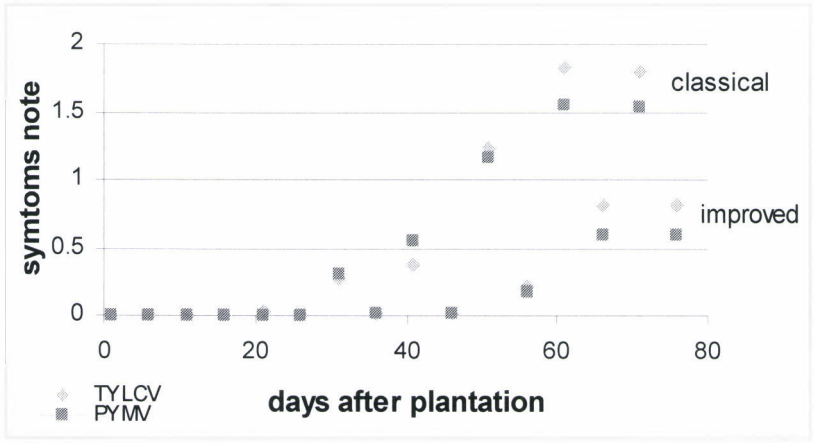
Results
Bemisia tabaci counting on yellow sticky traps



On the two graphs the number of *B. tabaci* is increasing up to 19 days after plantation on both packages. Then the number is increasing again on plots 4, 6 and 8 which are near from a sweet peper plot.

Virus symptom:
At the end of the nursery time and before the plantation, the ananlysis did not detected any virus (TYLCV or PYMV) on the two nursery (classical and insect proof).

During the cropping time:



The improved package is always less affected by both virus (PYMV and TYLCV) than the classic one : the infestation is delayed and the severity is less (note maxi 0.75 against 1.9).

Flowering date and fruits set
60% of the Heatmaster plants were flowered on july 12th and 20% of the Vyta plants were flowered on july 19th. On august 2nd, flowers and fruits are counted on the 4th first truths on 10 plants.
The number of flowers is the same for Heatmaster or Vyta, but the number of setted fruits is less on Vyta which is more attacked by Ralstonia solanacearum.

Number of plants affected by bacterial wilt
The number of wilted plant reached 40% for Heatmaster and 80% for Vyta.

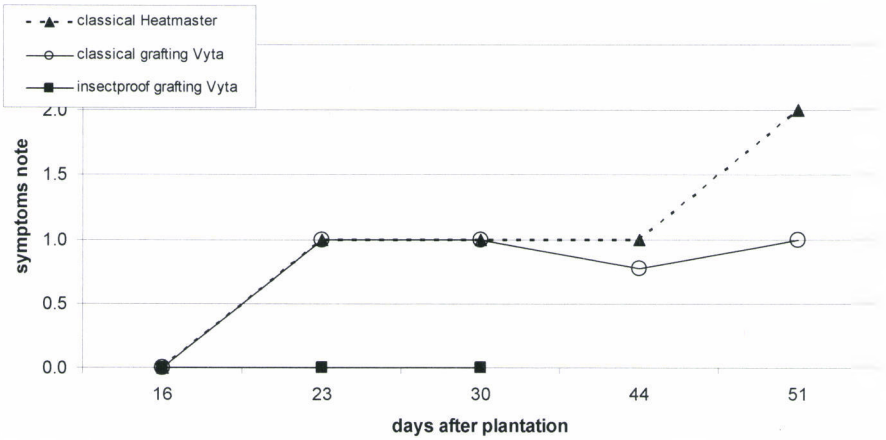
Evaluation of a IPM package during the rainy season
Materials and methods
Objects
classical package: variety Heatmaster (tolerance to bacterial wilt) grown 21 days under classical nursery;
improved package grafting: variety Vyta (tolerant to begomovirus) grown under classical, grafted on a rootstock resistant to bacterial wilt (Caraïbo).
improved package insect proof + grafting: variety Vyta (tolerant to begomovirus) grown under insect proof nursery, grafted on a rootstock resistant to bacterial wilt (Caraïbo).

Lay Out

Plots C3 and C4 (22m x 18m), 20 lines, space between lines 1.2m, density 2 plants/m2
Elementary plot: 5 lines of 20 plants, 100 plants in total. 4 blocs.
Sowing 26 september to 7 november, plantation 26 november. No pesticid was applied on the plot.

Results
Counting of B. tabaci
The number of *B. tabaci* is increasing for all objects up to the 23rd day after plantation (up to 40 adult on 10x10cm trap) and decreased after the 30th day. We did not observe any spatial repartition. The number of *B. tabaci* between the 3 objects in the day 23rd is not significant.
Virus symptoms:
At the end of the nursery time and before the plantation, the analysis did not detect any virus (TYLCV or PYMV) on the two nurseries (classical and insect proof).

During the cropping time:



From the 44th day, the object "classical Heatmaster" shows more virus symptoms than "classical grafting Vyta". The plants "insect proof grafting Vyta" have suffered in the nursery, only one line has been planted ; on this line no virus symptoms was present up to the 30th day, after that date all the remain plants died from collar rot.

Conclusions
Grafting of Vyta on Caraïbo has been successful under the classical nursery. The grafting done under the insect proof nursery have been affected by excess of temperature; even adding a shade net was not sufficient to reduce temperature. This technics of insect proof nursery needs some more attention for the hot areas.
The package "insect proof nursery + virus resistant variety + grafting on bacterial wilt resistant rootstock" was evaluated only one line of plantation, and no virus symptom was present on this line. So, if we solve the problem of temperature for grafting, this package is promising. The reduction of temperature may be obtain by misting and shading the nursery: this will be tried next year.
The package "classical nursery + virus resistant variety + grafting on bacterial wilt resistant rootstock" shows less virus symptoms than "classical nursery + bacterial wilt tolerant variety". These package may be a temporary solution to produce tomato in infected area.

5. Problem encountered
Experiment sites were not completely under control (it was a center of permanent training), therefore we got some problem to access to the site during vacation and data were lost.

6. Technology implementation plan
- During the years 2004 and 2005, the production of tomato decreased a lot in Martinica. A comity has been established where Extension service, Plant Health service, Coop technical service and the Cirad joined to analyse the situation and to propose some solutions.
- The situation has been analysed through weekly meeting during 3 months and different documents have been edited (there are presented at the end of the report).
- 5 technical sheets for the growers:
- Good Agricultural Practises

- Insects, mites and nematods
- Fungus, bacteria and viruses
- Begomoviruses on tomato
- Bacterial wilt on tomato
- One guide to IPM control of the vegetables pests by Cirad.

A general meeting has been held with the farmers in February 2006, the analysis of the situation has been explained, the different technical sheet presented and distributed and a wide discussion took place. Now the extension officers continue to visit the farmers and to explain the solutions proposed directly on the field.

7. Publications and papers

- 5 technical sheets for the growers, edited together with Cirad, Chamber of Agriculture, Plant Protection Unit of the Ministry of Agriculture, Cooperative SOCPMA, Federation Regionale de Defense contre les Organismes Nuisibles, 2005:
- Good Agricultural Practises
- Insects, mites and nematods
- Fungus, bacteria and viruses
- Begomoviruses on tomato
- Bacterial wilt on tomato
- Philippe Ryckewaert, CIRAD, 2005, A guide to IPM control of the vegetables pests

8. Conclusion

To propose an IPM package efficient against the damage of begomoviruses and *Ralstonia solanacearum*, several points need further investigations:

- how to lower the temperature under an insect proof nursery; for the hot area, misting should be considered.
- how to reduce the level of begomovirus inoculum in the environment ; as it has been showed that tomato is the main reservoir of the begomovirus, sanitary vacuum has to be organized at region level. This sanitary vacuum should be of around 3 months and should rotate around different region: this suppose to have a strong farmer organization.
- how to reduce the level of inoculum of Ralstonia solanacearum ; rotations have to be practiced, density to be decreased.
- conditions of grafting should be improved and should be practiced by the nursery specialist ; therefore, training is needed.
- varieties should be improved in order to combined both resistances to begomovirus and to *Ralstonia solanacearum*.

CIRAD MONTPELLIER – Michel Peterschmitt

1. Objectives

Bemisia tabaci is the unique known vector of all the begomoviruses. *B. tabaci* is generally considered as a species complex because, although morphologically indistinguishable, populations can be distinguished by host range, induction of silver leaf symptoms on *Cucurbita spp.* and efficiency to transmit viruses. Moreover, populations that are geographically isolated are not easily mating. This biological diversity motivated an identification of the *B. tabaci* populations present in the Caribbean islands. Comparison of the biological features between populations of different geographic origins was not feasible; indeed, to be reliable this comparison should have been carried out in a single place with living whiteflies of different geographic origin, requiring large containment chambers and an important manpower which was not available with any of the partners. Therefore, as morphological features are useless to identify *B. tabaci* populations, we needed molecular techniques.

The biotype B of *Bemisia tabaci* has spread throughout the world during the last 20 years and particularly to the American continent. In most of the places where biotype B was reported, an indigenous biotype was detected. Depending of the relative biotic potential and the difference of host range between the invading B biotype and the indigenous biotype, the indigenous biotype was either displaced as for example biotype A in north America, or remained in competition on the same hosts resulting in the persistence of both biotypes in the same environment as for example with biotype Q in Spain or North East Morocco.

Based on preliminary results where only biotype B was detected in the Caribbean, the displacement situation is expected like on the American continent. In the present project we suggested to confirm this displacement situation with larger samples and collected from the different Islands involved in the project.

2. Activities

A two-steps analysis was used to detect non-B biotypes in the Caribbean islands. The first step consisted in screening the populations with two microsatellite markers that enabled us to distinguish B and non-B individuals on agarose gels. The second step consisted in sequencing a fragment of the cytochrome oxidase 1 gene (CO1) of individuals that were representative of different microsatellite patterns; some individuals with the typical B pattern and several individuals exhibiting non-B patterns including biotype A pattern.

One of the two microsatellite marker, MS145, was selected among the microsatellite markers developed at CIRAD. With this marker, a product (allele) of about 230 bp is expected with a biotype B individual, and a product of about 190 bp is expected with a biotype A individual. The other microsatellite marker, Bem23, was developed by De Barro et al. (2003; Molecular Ecology Notes, 3 (1): 40-43). With Bem 23, a product of 230 bp is expected with a biotype B individual, but no product with biotype A.

As decided at the launching meeting in Guadeloupe in 2002, every partner has sampled *B. tabaci* populations and sent them to Montpellier for analysis. The number of individuals analysed from each island and the microsatellite patterns yielded are indicated in Table1.

Table1: Analysis of 271 individuals of *B. tabaci* collected in different islands of the Caribbean. The amplification patterns yielded on agarose gels with the microsatellite markers Bem23 et MS145 are indicated. For most of the patterns, a fragment of cytochrome oxydase 1 (CO1) gene was sequenced for one or more representative individuals.

	Total White flies	Amplification patterns(1)								
		1	2	3	4	5	6	7	8	9
		Bem23 **230**	Bem23 **230**	Bem23 **230**	Bem23 No product	Bem23 **230**	Bem23 **230**	Bem23 No product	Bem23 **230** product	Bem23 100 **240**
		MS145 **230**	MS145 **230**+ other product	MS145 No product	MS145 **230**	MS145 400	MS145 180 370 **440**	MS145 **190**	MS145 **190**	MS145 No product
Cuba	102	82	15	3	2					
Dominican R.	34	32	2							
Guadeloupe	37	29	7						1	
Martinique	35	33			1			1		
Trinidad & T	63	34	24	2		1	1			1
CO1 sequencing and biotype		4B(2)	10B	1B	2B			A	A	Distinct species ?

(1) The size of the products expected for biotype A and B are in bold

(2) Number of individuals of each pattern which were sequenced and biotype identified

Most of the individuals collected (210/277) were exhibiting the pattern obtained with control biotype B individuals (pattern 1) consisting of a product of 230 bps with Bem23 and 230 bps with MS145. These control biotype B individuals were from a rearing population from Guadeloupe. Two individuals from Cuba (5-249, 10-501) and two individuals of Trinidad (30, 80) exhibiting pattern 1 were used to confirm the biotype identification based on CO1 sequencing. Based on a neighbour joining tree comparing CO1 sequences, these 4 individuals clustered with biotype B individuals for which sequences are available in Genbank (Fig. 1).

A total of 48 individuals exhibited pattern 2 which consisted, a part from the 230 bps products of pattern 1, additional products for marker MS145. Five individuals from Cuba (1-6, 2-25, 3-95, 8-419, 12-171) and 5 from Trinidad (3, 5, 10, 44, 84) representative of pattern 2 were further analysed with CO1 sequencing. All the 10 individuals clustered with biotype B individuals in the phylogenetic tree (Fig. 1).

With 8 individuals the expected product of 230bp was only obtained with one of the two markers (patterns 3 and 4). Three individuals from Cuba representative of these patterns were further analysed with CO1

sequencing (4-204, 7-356, 9-489). All the 3 individuals were having a typical biotype B CO1 sequence (Fig. 1).

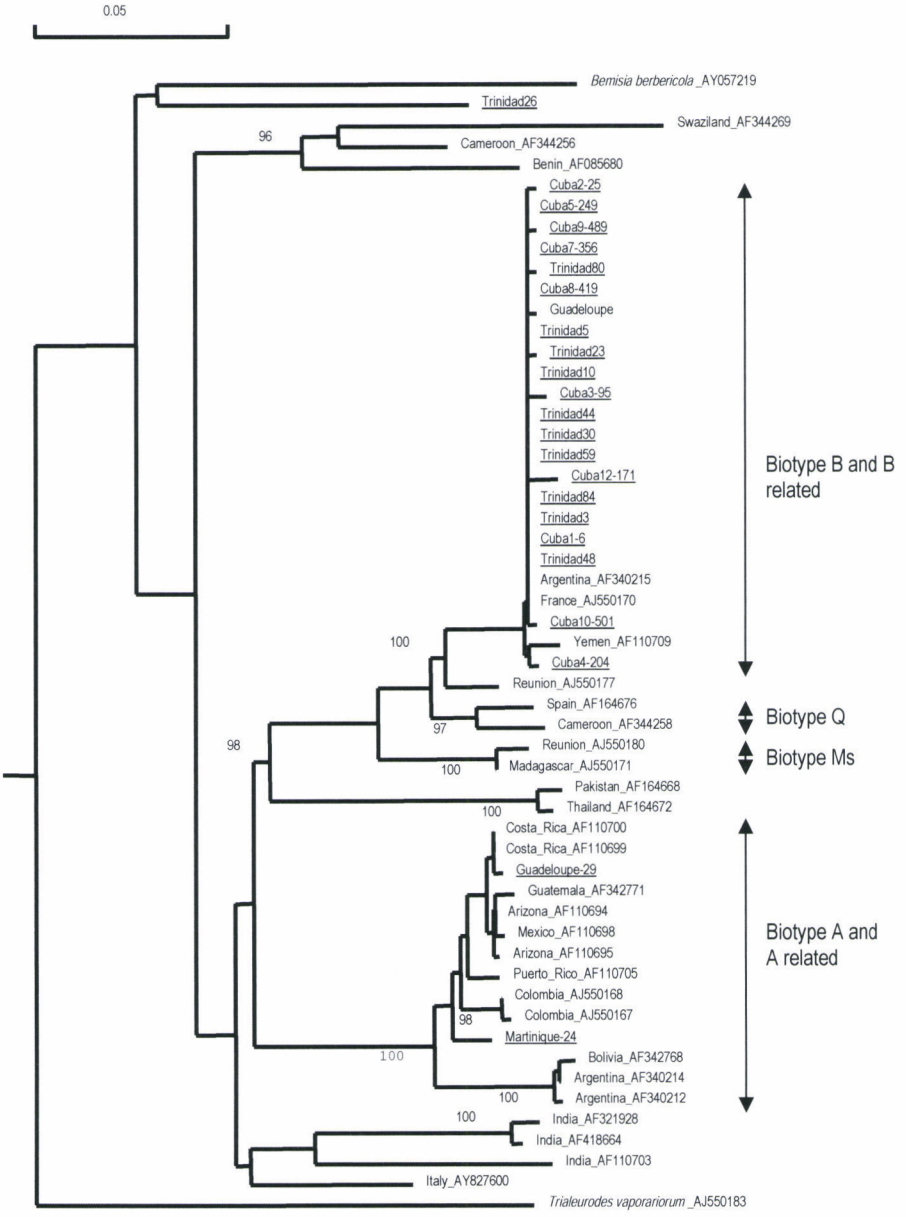


Fig. 1: Rooted neighbour joining tree showing the genetic distance among 370 nt cytochrome oxidase 1 fragments of *Bemisia tabaci*, either sequenced in this study (underlined) or selected from Genbank. The tree was set up with a Jukes and Cantor distance matrix using the Neighbour Joining method of DNAMAN. Sequences are identified by the country where the tested whiteflies were collected and by their accession number or sample number for the Caribbean individuals. Trialeurodes vaporariorum was used as outgroup. Sequences of Bemisia berbericola was added to this comparison to show the genetic distance between two species within the genus Bemisia. The scale measures the Jukes and Cantor distance between sequences. Numbers associated with nodes represent the percentage of 1000 bootstrap iterations supporting the nodes; only percentages ≥ 90 % are indicated.

Patterns 5-9 were obtained with one individual each. Individual exhibiting pattern 5 and the one exhibiting pattern 6 may be identified as belonging to biotype B because these patterns were similar to patterns 2-4 which were associated to biotype B based on CO1 sequencing of representative individuals.
Pattern 7 was obtained with biotype A control individuals from USA (J. K. Brown). The unique Caribbean individual exhibiting this pattern was from Martinique (24). Based on CO1 sequences, individual 24 clusters with biotype A individuals (Fig. 1). It had a typical biotype A CO1 sequence. Individual 29 from Guadeloupe also exhibited the expected 190bp product for biotype A with marker 145, but unlike the biotype A control, it exhibited a 230 bp product with Bem23 (pattern 8). Based on CO1 sequences, individual 29 clusters with biotype A individuals (Fig. 1).
Pattern 9, exhibited by individual 26 from Trinidad, did not consist of any of the products expected with biotype A or B. Based on CO1 sequences, the genetic distance between individual 26 and the *B. tabaci* individuals was almost as high as the distance between B. berbericola and B. tabaci. Individual 26 is either belonging to a distant biotype of *B. tabaci* or to a distinct species of whiteflies.

3. Results achieved
The collaboration with several Caribbean islands within the Betocarib project enabled us to expand our view on the populations of *B. tabaci* in Caribbean. Consistently with several reports on the introduction of biotype B in the US and the Caribbean Basin beginning in about 1986, the dominant populations in all the 5 islands were belonging to biotype B. Due to a larger host range and a higher multiplication rate of biotype B compared to indigenous populations, it was believed that indigenous populations were displaced. Since two individuals belonging to American cluster were detected in two Caribbean islands, Martinique and Guadeloupe, we conclude that indigenous populations were able to maintain on some host species. To our knowledge, this is the first report of indigenous populations of *B. tabaci* in the Caribbean Islands following the introduction of biotype B.

4. Problem encountered
No major problem

5. Technology implementation plan
Not applicable

6. Publications and papers
Peterschmitt et al. (in preparation). Detection of rare indigenous populations of *Bemisia tabaci* beside dominant populations of biotype B in the Caribbean Islands.

7. Conclusion
The benefit arising from this project regarding populations of *B. tabaci* is clearly the opportunity to carry out an extensive sampling throughout the Caribbean islands from the Northern Cuba Island to the most southern Trinidad and Tobago. A comprehensive view of *B. tabaci* populations was established in the Caribbean.
As described above, this is the first report of indigenous populations of *B. tabaci* in the Caribbean since the introduction of biotype B into the America Continent.

INDIVIDUAL PARTNER P2 FINAL REPORT

PARTNER 2: Natural Resources Institute – NRI (UK)
John Holt
Tim Chancellor

1. Objectives

The general objective of BETOCARIB was to develop integrated pest management (IPM) packages with the aim of increasing tomato production and sustainability of cropping systems in the Caribbean islands, where whitefly-transmitted begomoviruses threaten tomato crops.

How general objective was achieved	The roles of Partner 2, NRI
Increased scientific knowledge of the epidemiology of viruses transmitted by whiteflies within the tropical areas, combining the expertise of European and Third country research groups.	Models to explain begomovirus prevalence and challenge current assumptions both at the crop and the ecosystem level. Visits by a Caribbean partner (Cuba) to strengthen in-country modelling expertise
Identification of the key factors involved in epidemics and the development of epidemiological models.	Multiple regression and logistic models to distinguish those factors that have an association with begomovirus incidence and severity and to quantify the magnitudes of the effects. Simulation of within-crop disease progress to quantify the effects of specific management interventions
Based on the models, the establishment of IPM packages adapted to small plots growers limited resources within a year round tomato production system.	Of the factors which constitute potential management interventions, identify which are effective and should therefore be part of a package
Greater scientific and technical co-operation between the research institutions of the Community and the Caribbean, since the Lesser Antilles are not often involved in major projects concerning the Caribbean area.	Complementary skills in field work and modelling to produce technical advances and scientific outputs

The key activities cover 5 scientific research disciplines: biosystematics, epidemiology, plant screening, modelling and agricultural practices, which interact with each other to contribute to the development of IPM packages for begomovirus management in tomato crops, including risk management.
Partner 2 led one of the four workpackages (Epidemiology) and provided inputs within the disciplines : epidemiology, modelling, agricultural practices, and in the development of IPM packages

2. Activities

T02.01 Workshop Wo1 on relationship between agrosystems and epidemics, development of model

The first workshop was in part dedicated to the consolidation of knowledge on the factors influencing begomovirus epidemics. Invited speakers and extension workers participated and this helped to extend the range of experience brought to bear beyond the immediate project collaborators. The workshop was the starting point in the formulation of the agroecosystem model to predict begomovirus disease occurrence under different growing conditions and Caribbean environments.

T02.02 Target survey to validate the agroecosystem model

The epidemiological surveys provided the data for the statistical modelling used to establish which of the potential explanatory variables had an effect on the response variables, and so arrive at a minimum adequate model that contains all significant but only significant terms, as well as establish were differences existed between survey locations. The surveys in Guadeloupe were carried out, led by INRA, between 26/3 & 18/7 2002. A total of 84 and 101 fields were surveyed in Basse Terre and Grande Terre, respectively. The surveys in Cuba were carried out led by IHLD & CENSA between 20/1 and 16/7 2003 and a total of 224 fields were surveyed. The data were collected according to the survey protocol and due to local conditions the protocol was modified for the Cuba survey.

T02.04 Identification of biotic and abiotic (climatic) factors prevalent in epidemics

Separate models were developed to predict begomovirus disease incidence, severity and whitefly abundance. Models were developed for the two Islands of Guadeloupe, Basse Terre and Grand Terre, and for Cuba. Where common patterns emerged, these suggest general predictors of begomovirus problems which might apply over a variety of locations.

T02.05 Development of crop model for epidemics
The 'Crop Model' was intended to simulate disease processes within a crop or a series of crops within a locality. Unlike the 'Ecosystem model' which examined statistical relationships between variables within the ecosystem, the crop model was a dynamic model formulated using a set of linked differential equations. The model was used to investigate the potential efficacy of different management interventions by revealing the likely effect of such interventions on the underlying epidemiological processes. For example, in plots protected by cloth barriers, immigration of whiteflies is reduced. Immigration rate is one of the model parameters and so the relationship between immigration rate and disease progress in the crop was explored using the model. Field experiments provided three pairs of disease progress curves together with corresponding data on the relative abundance of whiteflies in each member of the pair :
1. with and without barriers (incorporating yellow insecticide strip) under low inoculum conditions (here the disease progress curves are based on symptoms only),
2. with and without barriers under high inoculum conditions (without insecticide strip)
3. separate curves for TYLCV and PYMV based on virus specific detection in a sample of plants (First assay, control plots only)

T03.03 Workshop Wo2 and design of IPM packages
The focus of activities under WP C was to synthesize relevant findings from the epidemiological studies in WP B and use this information to help develop integrated pest management packages. This was done through regular interaction with partners in WPs B and C and was finalised during the project workshop held in Havanna, Cuba in December 2004.

T03.04 IPM package evaluation
In a process initiated during Workshop 2 (Wo 2) and developed further in-country, trials were set up to evaluate IPM packages appropriate for the countries concerned.

3. Results achieved:
T02.01 Workshop Wo1 on relationship between agrosystems and epidemics, development of model
A comprehensive list of variables were identified that may have an effect on the epidemiology of begomovirus and the ecology of their whitefly vectors in the Caribbean. These were then prioritised and specified in more detail including how and when these variables should, and could, be measured. This formed the basis for the development of a survey protocol, a collaborative effort between INRA and NRI.
The ecosystem model was envisaged as an empirical, statistical model to be based on general linear modelling methods. Thus, the variables of interest, or response variables, i.e. begomovirus disease incidence, begomovirus disease severity and whitefly abundance might be explained by a combination of linear relationships with other (explanatory) variables. To gain an understanding of the differences between Caribbean locations, surveys were planned (so allowing models to be fitted) in five locations, two in Guadeloupe (Basse Terre and Grande Terre) and three in Cuba (east, central and west).

T02.02 Target survey to validate the agroecosystem model
The epidemiological surveys in Guadeloupe and Cuba were carried out according to plan, the variables to be recorded having been specified during the workshop Wo1. Surveys were carried out for the two islands of Guadeloupe, Basse Terre and Grand Terre and in the Eastern, Central and Western Regions of Cuba. The data entry was agreed, based on a spreadsheet protocol. It was decided to modify the survey slightly in order to make it more meaningful for Cuban conditions. Partner 2 staff met with Partner 6 staff in Cuba from 15-21 March 2003 providing an opportunity for an excellent exchange of experience.

The fit of these models to the disease incidence and severity observed in the surveys were good, especially for Basse Terre. The model predicting disease incidence in Basse Terre has an r-value of 0.93 indicating that the model explained more than 86% (r2) of the variation in the incidence data. With the Cuba data analysed using logistic regression, the model predicted the occurrence of begomovirus infection correctly 87% of the time. Thus, the models for both Guadeloupe and Cuba both performed very well for the prediction of begomovirus infection on an ecosystem scale.

T02.04 Identification of biotic and abiotic (climatic) factors prevalent in epidemics
In Basse Terre, some tomato production occurred at relatively high altitudes and such locations above about 550m where characterised by both low disease and low Bemisia abundance. Below 550m, both disease incidence and whiteflies showed wide variation. At extreme values, whitefly abundance appeared to have an impact on disease. For the majority of cases with more intermediate values, whitefly abundance was not a good predictor of disease. This suggests that any control measures directed at whiteflies would need to be able to reduce whitefly numbers to very low levels before they would be likely to have any significant impact on disease incidence.

A number of variables associated with tomato cultivation and management were linked to disease incidence. Fields were scored for 'management practices' by assessing principally three factors: drip irrigation, weeding and general plant health were recorded as 'good' or 'bad'. Nearly 70% of the fields in the 'poor' management category fell in the highest incidence category. Conversely, nearly 80% of the fields in the 'good' management category fell in the lowest incidence category. The reason that begomovirus disease incidence had such a strong inverse relationship with the general quality of tomato growing practices remains to be investigated. This result suggests that controlled experiments involving the relevant variables would be useful.

Physical protection of the crop was also an important variable. This was scored as follows: 1 if a greenhouse was entirely enclosed by plastic, glass or insect-proof net or the plot had well developed barrier rows; 2. if the greenhouse was not fully enclosed or the barrier rows were less well developed; and 3. if no form of physical protection was used. Those fields with the highest physical protection score always had the highest score for management practices. Physical protection was also carried out in nurseries in some cases and there was a link between the use of physical protection in field and nursery. For those fields were the highest level of physical protection was used in the field, it was also used in the nursery.

Requiring further investigation was an interesting inverse relationship between physical protection and chemical use. Those fields with the highest chemical use score, all had the lowest physical protection score. Chemical use was scored not only according to use, but also according to whether the insecticide was used correctly. Thus a score of 3 indicates proper use of pesticides. The results suggested that physical protection and chemical use represented alternative approaches used by farmers to begomovirus disease control.

The results show that three variables: good general management practices, physical protection in field and physical protection in nursery represent a package of measures which together were associated with low disease incidence. Although chemical protection had some association with lower incidence, its effect was quite weak. This may be partly due to the fact that chemical use appeared to be used as an alternative to the package of measures which was strongly linked to low disease incidence. The result suggests a need to clarify the impact of whitefly insecticides on Begomovirus disease incidence under controlled conditions.

For the Cuba data a logistic regression approach was used in which fields were simply coded as virus infected or not. This allowed a useful interpretation of the results in terms of odds (or risk) of begomovirus infection. The effect of region was examined by defining three categories, Western, Eastern and Central. Fields in the western region were 6 times less likely to be infected with begomovirus than fields in the eastern region. The 95% confidence intervals were wide, ranging from 1.2 to 31 times less likely. Central region fields were not significantly different from the Eastern Region. Oct to Dec plantings were 25 times less likely to suffer infection than Feb to July plantings.

Fields with a slope (as opposed to a lack of slope) were less likely to be infected but the effect was small and the reason is unknown. There was a suggestion that fields with barriers to Bemisia movement tended to be less likely to be infected but the effect was not significant. Sheltered cultivation however had a massive effective with infection being 39 times less likely than open field cultivation. In contrast, higher scores for Bemisia abundance incurred higher infection risk, the effect being significant but not very large. By far the most important contributing factor to infection risk was varietal resistance, with those varieties classified as resistant being 100 times less likely to exhibit begomovirus infection. If planting occurred in the later rather than the earlier months of either planting season there was an increased risk (3.8 times) of infection. Overall, the model predicted infected fields correctly 85% of the time (110/130 cases) and predicted uninfected fields correctly 90% of the time (85/94 cases).

Certain variables tended to be associated with each other, i.e. particular sets of conditions tended to occur in the same field. Cluster analysis was performed to see if the fields (as defined by the explanatory variables) fell naturally into groupings, and whether these groupings were associated with disease incidence and severity. Ten of the variables tended to be linked in the same fields, i.e. a field either had generally high scores or generally low scores for this set of variables. These variables were:
field physical protection, field sheltered cultivation, general management practices, varietal resistance, growing season, field chemical protection, previous virus hosts, host continuity, nursery physical protection, and nursery chemical protection. High scores for this set of variables appear to represent a package of physical and chemical protection of the crop in nursery and field, use of resistant varieties and good general management practices. Linked to this were high scores for Bemisia host continuity and previous virus hosts, suggesting this cluster represents fields of well-managed but intensive tomato production.

High scores for these variables (i.e. Cluster 1) were associated with low begomovirus disease incidence and severity. 76% (14/58) of fields in Cluster 1 were not infected whereas 73% (113/155) of fields in Cluster 2 were infected. 94% (65/69) of fields in Cluster 1 had disease severity scores of 1 or less whereas 61% (94/155) of fields in Cluster 2 has severity scores greater than 1. Clearly, therefore a group of variables was associated with begomovirus disease and because this group were correlated it was not possible to say which were of particular importance. The logistic regression highlighted sheltered cultivation and varietal resistance and these two variables gave the best model. However these variables were interchangeable with other members of the ten listed above to give alternative models which were nearly as good.

T02.05 Development of crop model for epidemics
The crop model was fitted to the results of two experiments which were all carried out at Godet, Grand Terre, Guadeloupe. The first (Assay 1) involved the use of whitefly-proof cloth barriers arranged in the form of a 1.5m high fence around the plot. In addition, on the inside of the barrier was a yellow strip impregnated with insecticide, designed to attract any and kill any whiteflies that circumvented the barrier and entered the plot. The control plot had no such barriers. In the control plots, virus specific detection was also carried out on a sample of 40 plants per plot, so disease progress where available separately for TYLCV and PYMV in the control plots of Assay 1. In the second experiment (Assay 2) the yellow insecticide-impregnated strip was not used. Estimates of primary inoculum were obtained from other whitefly monitoring activities, also carried out at Godet over the same periods. These provided weekly estimates of the number of plants infected after one week's exposure in the field together with immigrant whitefly counts from the plants, and from a mast trap.

Parameter values for the fitted model proved close to independent. Some interesting results were obtained for the virus acquisition and inoculation rates. An inoculation rate close to the theoretical value for TYLCV gave a good fit to the data (for both viruses) when this was combined with a very low acquisition rate. Without this combination of values it was impossible to obtain a model which fitted the data both in the early and later parts of the disease progress curve. The apparently very low acquisition rate might be explained by whitefly behaviour. If whiteflies tend remain on the same host plant rather than moving at random within the crop, then the contact rate between vectors and infected plants would be considerably lower than expected. The low acquisition rate can be thought of combining the probability of contact with an infected plant and the probability that virus is acquired if contact occurs.

Model optimisation was carried out by allowing all possible sets of model parameters to vary in pairs. No improvement on model fit could be obtained. It was postulated that the barrier would affect two model parameters: whitefly immigration and whitefly loss rate. Immigration was expected to be reduced because the barrier should reduce whitefly entry to the crop. Loss rate is less easy to predict; it might increase due to mortality caused by the insecticide-impregnated strips, or it might decrease because whiteflies are prevented from leaving the plot, in the same way as they are prevented from entering. In the event, it was found that to obtain a good fit to the barrier plot data, immigration had to be reduced about 12-fold but loss rate had also to be reduced by about 25%. Disease progress in the barrier plots was very much slower than the control plots it therefore appears that this occurred despite the barrier causing reduced whitefly losses from the plot. The effect of the barriers was therefore achieved due to their dramatic their dramatic over-riding effect in reducing whitefly entry to the plot by at least an order of magnitude.

The comparison between Assays 1 and 2 proved interesting because lacking the insecticide-treated strips, whitefly mortality within the barrier plots of Assay 2 was expected to be less. Indeed, in Assay 2, disease progress was actually faster in the barrier plots. A number of parameters were expected to differ between Assays 1 and 2. Whitefly immigrant number and infectivity would be expected to differ from the first experiment and the data from the primary inoculum monitoring suggested that whitefly immigration was higher during Assay 2 than Assay 1. Whitefly infectivity may have been slightly lower, however, because although more of the test plants became infected in the primary inoculum monitoring during the period of the second assay, infectivity calculated per insect was slightly less. In the barrier plots, because the barriers lacked insecticide-treated strips, it was also expected that whitefly loss rate would be lower than in Assay 1.

Under a range of different assumptions about whitefly immigration, the model fitted to the Assay 2 data gave similar predictions with the loss rate having to be reduced by about 60% to obtain a good fit to the barrier plot data treatment. It appears that barriers, without the insecticide treated strips, have the effect of decreasing whitefly loss rate to such a degree that the positive effect of reduced immigration to the crop is completely negated. Thus, barriers to whitefly entry to the crop represent very much a 'two edged sword'. Fewer may enter but are then retained resulting in very rapid disease spread.

The disease curve for PYMV alone was similar to that for the combined curve (based on symptoms) and the parameter values for the Assay 1 provided a good fit to the data for PYMV. The disease progress curve for TYLCV was slower than that for PYMV. Based on the assumption that no interaction existed between the two viruses, the theoretical incidence of single and double infections were calculated and compared with those observed. There was very close agreement between the two so there was no need to consider any interaction when modelling the progress curves.

Three parameters might be expected to vary between the two viruses, acquisition rate, inoculation rate and the infectivity of the immigrant whiteflies. Laboratory transmission tests showed TYLCV to have a higher inoculation rate than PYMV. Nothing is known about the respective acquisition rates but because the whitefly population in Guadeloupe has been in contact with TYLCV for a relatively short time, it may be more difficult for it to acquire TYLCV than PYMV. In the specific situation of this experiment it may have been that immigrant whitefly infectivity with PYMV was higher than with TYLCV. This is because there was another experiment close by with a large tomato plot deliberately infected with PYMV. It was found that different combinations of values of the three parameters concerned provided a good fit to the TYLCV data. The alternative the closest to prior expectation of how the parameters might change was a combination of higher inoculation rate, lower acquisition rate and immigrant infectivity the same as that for PYMV.

T03.03 Workshop Wo2 and design of IPM packages

The results of the ecosystem models suggested certain key components in begomovirus epidemics. Variables that were negatively associated with begomovirus disease incidence or whitefly abundance were particularly useful as most of these could potentially be exploited to improve management practices. These variables included the use of barriers and chemical and physical protection. The first two of these variables are being evaluated further in field trials in Guadeloupe and Cuba, respectively.

The barrier experiments have been described in detail above. The insecticide trial, which was jointly designed with IHLD, was conducted from December 2003 until March 2004 in Cuba (see Partner 7 report). Small plots containing fifteen tomato plants were used to assess the effect of applying imidacloprid in the nursery and in the field. Whitefly numbers were low on each of the two assessment dates (30 and 60 days after sowing) but begomovirus disease incidence was still high. Even at the first assessment date, disease incidence ranged from 73% to 77% symptomatic plants and there was no significant difference between treatments.

The results of the insecticide trial indicated that imidacloprid, as customarily applied by farmers in Cuba, was not effective in reducing whitefly numbers or disease incidence. It was probable that the protective effect of the insecticide was no longer in evidence at the time the tomato plants were removed from the nursery and planted in the field. Significant infection may have taken place during the period between transplanting and the application of Confidor at 35 DAS. However, it is not possible to establish this with certainty because the disease status of the tomato plants at the time of transplanting was not known. These findings are important because they suggest that current recommendations for use of imidacloprid in Cuba need to be revised. The effect of the seed treatment with Gaucho 70 WS lasts for a shorter

period than is stated by the manufacturers. Ideally, seed treatment should be combined with physical protection in the nursery and trials are needed to establish whether better results can be obtained by application of Confidor 350 SC earlier than is currently recommended.

Drawing on the findings from the various surveys and field trials, an attempt was made during the workshop in Havanna to group the variables associated with low or high begomovirus disease incidence (Table #). Interestingly, chemical protection, physical protection and good management practices were associated with low disease incidence in both Guadeloupe and Cuba. Similarly, other methods of exclusion, the use of barriers in Guadeloupe and sheltered cultivation in Cuba, were linked to low levels of disease. This classification was used as the basic framework for developing specific IPM packages for evaluation in Cuba and Guadeloupe. Firstly, schematic diagrams were drawn to illustrate the intervention points and the range of available options. Secondly, the diagrams were used to construct appropriate packages of control measures for inclusion in integrated management strategies in the respective countries.

Table #. Variables influencing geminivirus disease incidence on tomatoes in Guadeloupe and Cuba, based on survey data.

Guadeloupe	Cuba	Low/high disease	Common factor
Chemical protection	Chemical protection	Low	Yes
Physical protection	Physical protection	Low	Yes
Barriers	Sheltered cultivation	Low	(No)3
Management practices	Management practices	Low	Yes
Non-hosts (BT)1		Low	No
	Distance to edge (increasing)	Low	No
Hosts (GT)2	Previous hosts/host continuity	High	(No)3
Virus sources		High	No
	Susceptible variety	High	No

1 Basse Terre - 2 Grande Terre - 3 The variables are nevertheless closely linked

T03.04 IPM package evaluation

In both the greater and lesser Antilles (Cuba and Guadeloupe / Martinique, respectively), resistant varieties were chosen as the first component of an IPM strategy. The agronomic requirements and disease resistance characteristics of the varieties differ between locations and seasons. For example, resistance to Ralstonia solanacearum is required in Martinique and Guadeloupe, and in certain locations in Trinidad whereas it is not needed in Cuba. Unfortunately, the varietal screening conducted in Martinique was conducted under conditions of very low inoculum pressure and it was not possible to assess the resistance of the test lines and varieties to begomoviruses. Nevertheless, there are candidate varieties that are well adapted to the local environment which can be used in the IPM trials. The use of grafting will also be investigated in Martinique as this has the potential to address the twin problems of begomoviruses and R. solanacearum.

The experiments to evaluate the potential of grafting for improved disease management illustrate an important point about the design of the BETOCARIB project. As indicated above Work Packages B and C were designed to be mutually reinforcing and, even during the IPM evaluation stage, results from the management trials can usefully feed back into the crop modelling work. Thus, disease management experiments proposed for Cuba (insecticide trial) and Martinique (intercropping, mulches, grafting) can potentially be used for further crop modelling work. The outputs of the modelling will, in turn, allow the management recommendations to be re-evaluated and possibly refined.

4. Problem encountered

None

5. Technology implementation plan

The IPM packages which will include the use of tolerant/resistant plant varieties will be implemented in a number of representative Caribbean islands to determine their effectiveness. The most effective and sustainable IPM strategies will be transferred across the Caribbean, in literature and workshops form by means of the existing activities of the project Caribbean partners.

The potential exists to disseminate findings through regional organisations such as the Caribbean Agricultural Research and Development Institute (CARDI: http://www.cardi.org/), the regional research organisations under the Global Forum on Agricultural Research (PROCICARIBE: http://www.procicaribe.org/index.htm), and the Caribbean integrated pest management and vegetable networks (CIPMnet and CARIVEG, respectively). Another dissemination route is through the CGIAR system-wide initiative on IPM, and in particular the Tropical Whitefly IPM project led by CIAT (http://www.whitefly.org). With regard to EU initiatives, there is the European Whitefly Studies Network which has a newsletter (based at Rothamsted) as well as the Pesticides Initiative Programme (PIP).

6. Publications and papers

J. Holt, C. Pavis, T.C.B. Chancellor. Partially-protected cultivation to reduce the invasion of tomato crops by the virus vector Bemisia tabaci: modelling the impact on virus disease epidemiology (draft publication prepared).

A paper is also in preparation describing the results of the epidemiological surveys in Guadeloupe and Cuba.

7. Conclusion

New knowledge of begomovirus disease epidemiology in the Caribbean establishing and quantifying both the risk factors for disease and contributions of different disease amelioration measures.

Inter-island comparisons enabled generalities and differences in the conclusions to be determined. The findings of the project feed through directly as disease management advice which can be tailored to tomato growers in the different islands involved in the project as well as allowing extrapolation with a fair degree of confidence to other Caribbean locations.

Complementarities between project partners were very productive and strengthened scientific endeavour for all. The NRI perspective was that the project was a success story.

Future work might usefully further exploit the modelling tools that have been developed. In particular the crop model could be used to quantify the underlying epidemiological effects of other management interventions and so provide a rational basis for further improvements in practice.

INDIVIDUAL PARTNER P3 FINAL REPORT

PARTNER P3: Institut National de la Recherche Agronomique INRA Guadeloupe (France)
Claudie Pavis

1. Objectives

Partner P3 was partly involved in WPA (biosystematics), where its goal was to evaluate the transmission rates of the tomato begomoviruses occurring in Guadeloupe.
Partner P3 was mainly involved in WPB (epidemiology).
In order to determine the key-components of begomovirus diseases in the Caribbean, two islands were chosen to carry out target survey in tomato producers: i) Cuba for extensive tomato production systems, with large areas of production, and with at least two begomoviruses attacking tomato plants, and ii) Guadeloupe, more representative of the Lesser Antilles, with different pedoclimatic conditions, with small tomato parcels and with two tomato begomoviruses.
Our first objective was to carry out this survey in the different tomato production areas in Guadeloupe.
Secondly, the objective was to observe the disease progress in experimental plots. This objective was shared with other partners (P1, P6 and P7), in order to bring data from different ecosystems to partner P2, in charge of the epidemic modelling. These experiments were also designed to allow an evaluation of some components, stressed by the target survey, on the disease progress.

2. Activities

Transmission tests
To determine the transmission rates of the two viruses, we developed a standardised methodology of vector inoculation. This was achieved using synchronised whitefly rearing, acquisition on TYLCV or PYMV-infected tomato plants, transmission on caged tomato plantlets with batches of 3, 10 and 25 whiteflies, symptom observation, and ELISA detection. Fifty replicates were made with each virus and number of inoculating females.

Target survey
During the first meeting, we organised a brain-storming to determine the potential key factors which may have an effect on the incidence and the severity of the tomato begomovirus disease in the different situations.
Then, these potential factors were used to build the survey sheet. We tested a prototype of the survey sheet, and modified it. We proposed the survey sheet to the Cuban partner, who used it with a few adaptations.

In Guadeloupe, we carried out the survey at the beginning of the project. We focused on the two major tomato production areas, in which we visited a great part of the patented growers at a given period (estimated to 90%). About 200 plots were surveyed. Each grower was interviewed, to get information on cultural practices; incidence and severity were assesses in each plot, and the vector population was estimated by counting.
At the end of the survey, we sent the data to partner P2, we used it for modelling purpose. On the other hand, we analysed the data, which allowed us to describe the actual tomato production context in Guadeloupe.

Experiment on disease progress
After analysis of the target survey, some factors appeared to may have a significant impact on disease incidence. We carried out field experiment, under natural infection, in order to describe the disease progress in different situations; this was achieved by 2 trials in 2003 (800 and 600 plants respectively), 2 trials in 2004 (800 plants each), and one trial in 2005 (648 plants) with weekly symptom observations, trapping of whitefly with yellow sticky traps, and specific detection. Previously to the trial, we had to develop monitoring systems in order to quantify the primary viral inoculum, and the vector populations at the field station scale, to assess their impact on the disease progress; this was achieved using whitefly trapping systems, and batches of tomato plants in containers periodically let in the field as "virus trap".

3. Results achieved

The transmission tests showed that TYLCV had a higher ability to be transmitted to tomato than PYMV.

The target survey allowed to have a precise knowledge of the cultural practices in tomato growers of Guadeloupe, and gave information on parameters linked with high or low disease incidence. The pattern was unclear concerning the impact of disease severity, or on whitefly populations.
Low disease incidence occurred in the following situations:

- plots partially surrounded by physical barriers, such as hedges, sugarcane plots, etc.
- plots close to other crops, non-host of whiteflies
- plots in areas higher than 400 meters.

High disease incidence was linked with:

- plots close to other infected tomato plots
- plots with old plants

In the field experiments, we obtained the following results:

- The temporal disease progress fitted with a logistic model. The disease progress was parallel to the number of trapped vectors until 6 weeks after planting, then decreased. The incidence reached 100% after 2 month, which corresponds to very strong epidemics.
- The cultivation of tomato during a period of low inoculum pressure allowed avoiding symptoms during more than a month.
- The presence of physical barriers impregnated with insecticide added 2 weeks to this delay of symptom apparition. During the high inoculum pressure period, the barrier did not play a role on the epidemics.
- The two viruses (PYMV and TYLCV) progressed similarly in the plants, and a majority of plants had mixed infection at the end of the trial.
- The results of trials carried out with tomato mixed with coriander, supposed to reduce the disease incidence were unclear, due to problems of competition between the two crops.
- The results of trials carried out with a TYLCV-resistant variety vs a susceptible one were unclear, due to very severe natural infection: both varieties were attacked by PYMV and TYLCV.

4. Problem encountered
No major problem

5. Technology implementation plan
Not applicable

6. Publications and papers
Congrès

Pavis C., C. Urbino, L. Rousseau, D. Lange, N. Robin, A. Huc, R. Gotin & N. Boissot (2005). How epidemiological sudies on the begomovirus diseases of tomato may help to develop new cropping systems ? Meeting of the Caribbean Food Crop Society, Gosier (Guadeloupe), juillet 2005. Oral presentation.

Pavis C., C. Urbino, M. Marquier, J. Agrapart, N. Robin, L. Rousseau, D. Lange & N. Boissot (2005). Epidémiologie des maladies à Bégomovirus sur la tomate en Guadeloupe. 10èmes rencontres de virologie végétale, Aussois, 6 au 10 mars 2005. Poster.

7. Conclusion

INDIVIDUAL PARTNER P4 FINAL REPORT

PARTNER P4: University of West Indies – UWI (Trinidad)

Pathmanathan Umaharan

1. Objectives
The main objectives were to have a better knowledge of the prevalence of begomoviruses and Bemisia in Trinidad and Tobago and to develop adapted integrated pest management.

2. Activities
The participation to WPA was defined during the first meeting in Guadeloupe and applied afterwards to precise the biosystematics of both begomoviruses and bemisia. IPM practises including evaluation of varieties were set up the last year as defined in WPC.

Tomato production systems:
The study was based on an island-wide farmer survey conducted in 2002, which identified the major tomato growing areas and the associated problems in the areas. A total of 112 tomato farmers and 55 extension workers were interviewed.

Biosystematics of begomovirus and Bemisia
Potato yellow mosaic Trinidad virus (PYMTV) was the only begomovirus detected in Trinidad and Tobago based on an epidemiological survey conducted in 2002 and again in 2004/05 using DNA sequence analysis of a 1.1 kb fragment of DNA-A amplified by IC-PCR. IC-PCR based (Rampersad and Umaharan, 2003a) and standard-PCR based methods (Rampersad and Umaharan, in press) were developed as a diagnostic tool to detect begomoviruses from both tomato plants and weeds during this project.
Similarly, an extensive survey of *Bemisia tabaci* within tomato cropping areas as well as outside tomato growing areas was conducted. *Bemisia tabaci* biotype detection was carried out by RAPD analysis, sequence analysis of PCR amplified segment of the COI gene and through SSR analysis (conducted in CIRAD).

Epidemiology of PYMTV
An epidemiological survey was conducted in two contrasting agro-ecosystems in Trinidad (a) Bonne Aventure (hilly, medium sized (5 ha), dispersed fields, tomato main crop, mainly single season cropping surrounded by sugarcane areas) with high begomovirus pressure and (b) Aranguez (flat terrain, small farms (< 1ha), contiguous cropping areas, vegetable including tomato, tomato grown continuously).

Identification of resistance to PYMTV
Thirty-two varieties obtained from the breeding programmes by Hazera Ltd, J.A. Scott (Florida against ToMoV), Olimpia Gomez (Cuba) were evaluated under natural epiphytotics under field conditions over three trials supported by ELISA to identify possible sources of resistance to PYMTV.

IPM study
Highly resistant- moderately resistant- and susceptible varieties were compared under two IPM regimes (no IPM vs IPM based on the epidemiological study) in a split plot design. The IPM methods employed were nursery chemical and physical protection, and the use of imidacloprid at planting and two weeks later as a drench

3. Results achieved
Scientific results
Begomoviruses were the most important problem in all tomato growing areas and Potato yellow mosaic Trinidad virus (PYMTV) was the only begomovirus detected in Trinidad and Tobago. Bacterial wilt was also a problem in specific tomato growing areas (Umaharan, 2003).
An ELISA survey showed that PYMTV was epidemiologically the most important virus in tomato production systems in Trinidad. Where tomato is grown with other crops, Tobacco etch virus and Tobacco mosaic virus were detected albeit at lower incidences (Rampersad, 2004). Two surveys were conducted the first in tomato growing areas around Trinidad and the second in tomato non-growing areas. Both did not reveal the presence of PYMTV in none of the weeds sampled (Rampersad and Umaharan, 2003b). Two other bipartite begomoviruses were however identified in Rhyncosia and Calapogonium (Rampersad and Umaharan, 2003c).

Only Biotype-B was detected in the survey (This would be published soon).
In Aranguez that represented an area of high begomovirus pressure, the incidence reached saturation 50 days after planting regardless of a number of variables. Approximately 35% of the variation in severity was explained by the X variables nursery physical and chemical protection and plant health. This suggested that good nursery practices can greatly reduce the severity of the disease, possibly because it delays infection. Therefore in Aranguez some crop loss could be avoided by good nursery protection and good cultivation practices but varietal resistance should form the core of IPM.
In Bonne Aventure, barriers presented by distance between fields, field chemical protection, proximity of field to virus and Bemisia host fields as well as varietals susceptibility explained a large proportion of the variation in disease incidence. The results suggested that with good practices disease saturation can be delayed to as long as 80 days in Bonne Aventure. These results suggest that in Bonne Aventure, barrier crops and chemical protection can provide some relief but varietals resistance is important (Caruth, 2007 – M. Phil thesis).
Among the varieties tested, only H-3018 was immune to PYMV - both free of symptoms and free of virus in ELISA tests. There were however a number of varieties with useful levels of resistance H-3105, H3108, Cuba-12, Cuba-32, Cuba48 and Cuba 57 to begomoviruses. These moderately resistant varieties showed a significantly lower percentage incidence and a lower severity score at 60 days after planting compared to the susceptible varieties (>95% incidence) (Walker, J, 2007 – M.Phil thesis).

Technology development
Due to lack of time and resources the IPM measures were tested only in one location during one season. It is intended that this experiment will be repeated in collaboration with the Ministry of Agriculture at multiple locations and over two seasons. This will allow fine turning of recommendations to each location/ season.
IPM measures delayed disease incidence in the moderately resistant varieties to <20% at flowering, but did not affect disease severity. The highly resistant category and the susceptible varieties did not benefit from the IPM measures. Among the susceptible category the indeterminate varieties suffered less in terms of yield compared to the determinate varieties. The results showed that yield losses can be effectively minimized by using either resistant varieties or moderately resistant varieties supported by IPM.

The tomato varieties were also assessed for fruit quality, productivity and for their tolerance to other bacterial and fungal diseases. Based on these the following varieties were identified for release (Table-1)

Table-1 Tomato varieties recommended for the various seasons in Trinidad and Tobago

Varieties	Origin	Conditions	Comments
HA3018	Hazera	Open field	HA3018 is immune to PYMTV
Cuba 57	Cuba	(Early wet & late wet)	Cuba 57 show low disease incidence and severity score, stable yields; both susceptible to bacterial wilt.
HA3105, HA3108	Hazera,	Open field (Early wet)	Low disease incidence and severity score, stable yields; both
Cuba 48, Cuba 12	Cuba		susceptible to bacterial wilt.

4. Problem encountered
No major problem

5. Technology implementation plan
A national symposium will be organised in June 2007 to disseminate the information derived from the project and the recommendations. Researchers, extension workers, agrochemical/ seed companies, farmers, nursery growers and other stakeholders will be invited.

A pamphlet will be produced for distribution by the extension division of the Ministry of Agriculture. The pamphlet will outline: recommended varieties; Chemical and Physical protection of nurseries; appropriate use of imidacloprid at planting; synchronous planting where possible or use of barrier crops / trap crops; removal and destruction of crop immediately following harvesting; methods of monitoring whiteflies.

A training of quarantine officers to improve surveillance to reduce the chance of introduction of Tomato yellow leaf curl virus (TYLCV), which is a more devastating begomovirus than PYMTV, into the country. The training will involve greater awareness of the threat as well as hands-on training on serological and PCR-based detection methods.

Caribbean Chemicals Ltd a company specializing in the importation and distribution of tomato seeds in the Caribbean region has already been approached. The company is setting up pilot farmer field plots in the major tomato growing areas to demonstrate the new varieties to the farmers. The company is also planning to enter into a contract to import hybrid seeds from Cuba and Hazera Ltd. The University of the West Indies will provide technical support.

A field day will be conducted at the University of the West Indies to demonstrate to farmers the shelter production of tomato. We are already in the process setting up new shelter houses for this purpose.

The University will also include a webpage on begomoviruses and Bemisia management in tomato on its outreach website. This will allow ready access to farmers around the country to access information in tomato. (This is already underway).

A farmer survey will be conducted to determine the adoption rate two years after implementation.

6. Publications and papers

Rampersad, S.N. and Umaharan, P. (2005). The Effect of Plant Genomic DNA on the Detection of Mixed Infections of Begomoviruses using PCR with Degenerate Primers. Tropical Agriculture (in press).

Rampersad, S.N and Umaharan, P.(2003). Identification of resistance to PYMV-TT among accessions of Lycopersicon spp. Plant Disease. 87 (6): 686-691

Rampersad, S.N., Umaharan, P. (2003) Detection of Begomoviruses in Clarified Plant Extracts: A Comparison of Standard, Direct-Binding, and Immunocapture Polymerase Chain Reaction Techniques. Phytopathology 93 (9): 1153-1157.

Rampersad, S.N., Umaharan, P. (2003). Detection of Two Bipartite Geminiviruses Infecting Dicotyledonous Weeds in Trinidad. Plant Disease, 601- 602.

Umaharan, P (2003). A survey of begomovirus and whitefly management in Lycopersicon esculentum L. cultivation in Trinidad and Tobago. Technical paper. Department of Life Sciences, The University of the West Indies, St. Augustine, Trinidad.

In preparation:

1. Characterisation of *Bemisia tabaci* populations in the Caribbean
2. Epidemiology of PYMTV in two contrasting ecosystems in Trinidad and Tobago
3. Identification of resistance to PYMTV in tomato
4. Molecular characterization of PYMTV variation in Trinidad and Tobago
5. Effect of PYMTV on yield and fruit quality in tomato
6. Mechanism of tolerance to PYMTV in tomato

Thesis

Caruth, C (2007). Molecular genetic diversity of PYMTV and *Bemisia tabaci* in Trinidad and Tobago and the epidemiology of PYMTV disease. Writing up.

M.Phil thesis - Walker, J (2007). Effect of PYMV on tomato yield and quality in 22 varieties of tomato (Lycopersicon esculentum). – writing up

PhD thesis – Rampersad, S (2004). Molecular and Genetic characterisation of geminiviruses in tomato and in alternate weed hosts. The Library, the University of the West Indies, St. Augustine campus, Trinidad and Tobago.

7. Conclusion

Additional work is necessary to develop new varieties that combine resistance to begomoviruses with bacterial wilt. There is already work underway in INRA, Guadeloupe on the above topic.These varieties should be screened for PYMTV resistance.

A shuttle breeding programme should be instituted to develop durable resistance to begomoviruses in the Caribbean. A collaborative genetic study will allow the identification of complementary resistant genes that should be incorporated.

INDIVIDUAL PARTNER P5 FINAL REPORT

PARTNER P5: Instituto Superior de Agricultura – ISA (Dominican Republic)

Pedro Benoit

1. Objectives

General context:

By the beginning of the 90´s, the processing tomato production in the Dominican Republic was strongly affected by TYLCV, having *Bemisia tabaci* as a vector. That situation caused yield reductions from 30 to 100%, the lost of millions dollars to the country economy, and no supply of tomato byproducts were available at the local market, so importation of processed tomato was required, in order to satisfy the national demand. As a problem of national concern, the Ministry of Agriculture together with private sector (research centers, processing enterprises), developed strategies to reduce the impact of TYLCV in tomato. As a consequence of this, new alternatives were obtained, like for example, the introduction of a no host period where no specie hosting *B. tabaci* is allowed to grow during the period July 1 to October 1. With this, a remarkable reduction in the *B. tabaci* population has been obtained with the positive effect of less problems of TYLCV at the beginning of the crop season.

As part of the collaboration to find a solution to that problem, during the period 1992 to 1994, the Instituto Superior de Agricultura together with the Junta Agroempresarial Dominicana (JAD) and Transagricola (a processing tomato enterprise), worked in a project to study the short time hosts of geminivirus in tomato, transmitted by whitefly, including the dynamics of the population of the vector and its natural enemies, in the northern and southern regions of the country.

Objectives of the participation in the BETOCARIB Project-:

Due to the involvement in researches concerning to TYLCV and *Bemisia tabaci* in tomato, ISA accepted to participate in the BETOCARIB Project, in order to collaborate with the other colleagues from the Caribbean Region, sharing the experiences in crop management, conducting field surveys and establishment of strategies for IPM packages.

Another reason of the participation in the Project is the accumulated experience in the management of the problem, by research institutions, companies and many tomato growers, where ISA could help as a intermediate or contact for the other countries involved in the Project.

2. Activities

The main activities carried out by ISA have been the field surveys to collect samples of tomato leaves (for virus detection and identification) and sample of *Bemisia tabaci* (for characterization). Those activities were done during the first and second year of the Project. Collected samples were sent to France and Cuba.

During the four years of the Project, field evaluations were conducted and the beginning and by the end of the crop season, in order to evaluate the population of whitefly in the fields and the presence of symptoms caused by TYLCV. All this was done in processing tomato farms in the north and south regions of the country, and in some salad tomato plantations in the north region.

3. Results Achieved

Results of the Year one (02/01/02 to 01/31/03)

During this period, contact with technicians and tomato growers, were made, in order to establish the areas where surveys and sampling will be done. Also contact and first meeting with BETOCARIB members was held in Guadeloupe, for the integration in the Project and the assignation of Tasks.

Results of the Year two (02/01/03 to 01/31/04)

During the period January – February, 2003; Samples of tomato leaves and Bemisia tabaci, were taken from different tomato plantations in the north and south regions. *B. tabaci* samples were sent to France and tomato leaves sample to Cuba via Guadeloupe. Although the processing tomato production was not really affected by TYLCV during the period 2002/003, the surveys done at the beginning of the year showed a relative high level of infestation.

According to the results, the amount of *B. tabaci* samples was not sufficient for an adequate evaluation and the samples sent to Cuba were in very bad conditions because problems with the delivery of plant material between countries.

Also a second meeting was celebrated in Trinidad, where results of the previous activities were discussed and evaluated.

Results of the Year three (02/01/04 to 01/31/05)
According to the results of the tests conducted at CENSA (Cuba), using three different primers for the same leaf tomato samples, approximately 10% of the samples gave positive reactions to TYLCV. When Brown Primers were used, the amount increased to 36%, and when Rojas Primers were used, only 13% yielded positive reaction. Due to variations in the results, it seems to be that the amount of material analyzed was not sufficient to confirm the presence of TYLCV in the material.
In a second chance to deliver more material to Cuba,it was not possible to do the delivery because of international delivery restrictions for vegetative material.
In the case of the *B. tabaci* samples delivered to France. Practically all the whiteflies showed a typical biotype B profile. There were only three individuals showing a different pattern, needed to be confirming lately.

Results of the Year four (02/01/05 to 01/31/06)
Only field surveys were conducted in the north and south regions of the country, in order to evaluate the population of *B. tabaci* and the incidence of TYLCV in processing and salad tomato. According to the observations, the amount of Bemisia in both regions was in average less than 1 insect per plant, and the incidence of TYLCV was less than 3%.

4. Problems encountered
The main problems encountered were:
The delivery of the tomato leaf samples to the corresponding Partner; because of the international restrictions to the movement of plant material to another country.
The financial inconvenient, due to the delay to transfer the fund from the Commission that avoid us to attend the meeting in Cuba in November, 2004
The no fulfilment of the IPM part, as it was established at the beginning of the Project.

5. Technology implementation plan
The implementation plans of technologies have been established by the Ministry of Agriculture, together with the processing companies, international scientific support and tomato growers. Those plans include: a non host period of 3 months, selection of resistant varieties or hybrids, growing plantlets under protected conditions and use of specific insecticides.

Through surveys we evaluated how those measurements were taken into consideration by the tomato growers, in terms of the presence of whitefly in the fields and the level of TLCV incidence. Together with our colleagues identification and characterization were done, in order to verify the type of whitefly and begomovirus present in our country.

6. Publications and papers
No technical publication was made during the whole project.

7. Conclusion
We think that this was an excellent opportunity of collaboration between countries and institutions that gave us the chance of working with Scientifics in the area of plant protection. Although our participation was not so intensive as it was for the other countries, it was also an opportunity to initiate an international collaboration in the Caribbean Region.
For us the most important thing is that we know each other, so we can keep in contact for specific Project that we can develop in the future. Another aspect is the amount of new information that is already available for our countries.

INDIVIDUAL PARTNER P6 FINAL REPORT

PARTNER P6: National Center for Animal and Plant Healthy – CENSA (Cuba)

Yamila Martinez

1. Objectives

WPA Identification of begomoviruses and their vector affecting tomato crops in the Caribbean island and developments of tools for their specific diagnosis and identification of wild host species.
WP B Epidemiology and modelling to investigate the appropriate mix of disease control tools at both crop and ecosystem levels. Activities:

2. Activities

Samples Collect: We are doing two national surveys for collect 737 tomatoes, 25 between pepper, bean and weeds samples. We were colleted too 102 *B. tabaci* individuals from different host and localities.

Variability of begomovirus

DNA extraction from plant: We were the short methods in hot conditions as Quiñones et al, 2002. All samples with important results were purified using the protocol of Dellaporta, 1993.
Nucleic Acid hybridization-no radioactive (NAH): the NAH was done using the procedure write Quiñones et al, 2002.
The pre hybridization and hybridization procedures were carried out using a commercial solution contained the labelling kit, NaCl 0.5M and the blocking agent's 0.2% (content in the kit), according to the conditions described by Potter (2001).
The NAH using generic probe (PCTY) was carried out under conditions of low astringency at 42ºC, with the objective of detecting all the begomovirus present in the samples. The membranes were pre hybridized during two hours. The NAH with the specific probe (RITY) was carried out under conditions of high astringency to 65ºC and the membranes were pre hybridized during one hour. The hybridization with both probes was carried out during the whole night, at 65ºC for the case of the probe RI and at 42ºC for the probe PC.
Two wash were carried out in 20 minutes each one, with a solution that contained Urea 2M, SDS 0.1%, NaCl 150mM, blocking reagent to 0.2%, MgCl2 10mM and NaH2PO4 50mM, followed the incubation at 65ºC for the membranes hybridisation with the probe RI and at 42ºC for the hybridization with the probe PC, followed by other two wash to room temperature, during 10 minutes, in a solution of Tris1M, NaCl 2M and MgCl2 2mM, for both probes. Detection was carried out using CDP-star (dioxetano) reagent, content in the kit.
We used two probes: Probe 1, with size of 1.1Kb that include Rep N –Terminal region, intergenic region (IR) and 5' extreme from coat protein. Probe 2, with size of 797pb from intergenic region from TYLCV-Isr, isolated in Cuba.
Polymerase Chain Reaction: Two primers couple were used 1) 1978/496 specific for amplification of genome A of bipartite begomoviruses and 2) 800/3010, for genome B, both couple primer were uses according to Rojas et al ., 1993. We used too one primer for specific amplification of TYLCV-Isr intergenic region as Martínez et al., 2002.

Variability of *B. tabaci*

DNA extraction: We were used two methods for the DNA extraction.
1) From 102 *B. tabaci* individuals (Table I.1), according Delatte et al (2005). The principal steps are the selection an only insect and to deposit it on the filter paper to dry it, later on to transfer it to a PCR plaque with 10 µl of the extraction buffer, is important to conserve the plaque on a frozen surface to avoid evaporation, to macerate during 20 seconds with a pipette of sterile rounded tip, to add 15 µl of the extraction buffer (composition). We seal the plaque with an aluminium paper and we incubate 1 h at 65ºC and 15´ at 95ºC, after the incubation we add 35 µl of pure ultra water and to conserve at -20ºC until being used.
2) DNA from 680 *B. tabaci* individual insects was purified according Froit et al 1999 microsatellite maker: We selected DNA from different host and place (table I.1), for amplification the locis Bim 23 using primers BEM23F / CGG AGC TTG CGC CTT AGT Cy BEM23R / CGG CTT TAT CAT AGC TCT CGT (De Barro et al 2003). The results were compared with the control profile for biotype B, Q, A (from Dr N. Sauvion, CIRAD Guadalupe and Dr J Brown, Arizona, USA).
The results were observed in agarose 3% and observed using BET in transilluminator.

Amplification of cytochrome oxydase gene-1 (mtCOI): 12 DNA were selected from insects of different localities with the objective to compare the results obtained in microsatellite analyzed and to detect the molecular variability. The primers C1-J-2195/L2-N-3014 for mtCO gene were used according to Frolich et al, 1999 for studies of the variability in Bemisia population.

Table I.1. *B. tabaci* individual insects analyzed by microsatellite methods.

B. tabaci isolated	Province	Locality	Crops
PR3; PR6; PR8; PR10	Pinar del Río	Los Palacios	Squash in Sweet potatoes
PR13	Pinar del Río	Los Palacios	Tomato and cabbage
PR22; PR23; PR24	Pinar del Río	Los Palacios	Sweet potatoes
LH25; LH27	La Habana	Guines CAI O. Sánchez	tomato
LH460; LH489; LH511	La Habana	Guira	tomato
LH501; LH503; LH505	La Habana	Guira	Dasheen
G33; G34; G40; G50; G57	Granma	UBPC 28 de Enero. Veguita. Yara.	tomato
G52; G53	Granma	UBPC 28 de Enero. Veguita. Yara.	Malvaceae
G65; G72	Granma	Bayamo. La Pupa. C Protegidos.	tomato
G76; G95	Granma	Cauto Cristo. ECV. M de Artemisa	Pepper
G85	Granma	Cauto Cristo. ECV. M de Artemisa	cabbage
G90; G89	Granma	Cauto Cristo. ECV. M de Artemisa	tomato
H100; H101; H111; H118; H120	Holguín	Gibara. Margodo	tomato
H125; H127; H135	Holguín	Giquima. Huerto de C Protegidos.	Squash
H139; H149	Holguín	Biola. Rafael Freire	tomato
LT155; LT158; LT178; LT181	Las Tunas	ECV Las Tunas	tomato
LT166; LT508	Las Tunas	ECV Las Tunas	cucumber
LT171; LT172	Las Tunas	ECV Las Tunas	Pepper
C185	Camaguey	Camalote. Huerto de C Protegidos	tomato
C190	Camaguey	Camalote. C Protegidos	cucumber
C194; C102; C104; C204; C207;C213	Camaguey	ECV Camaguey. C Protegidos	tomato
C220; C224	Camaguey	Camalote. C Protegidos	tomato
C227; C230	Camaguey	ECV Camaguey Campesino	tomato
C237; C238; C241	Camaguey	ECV Camaguey Campesino	Malvaceae
C249; C253; C257	Camaguey	Florida.CCS Sabino Pupo	tomato
C263; C266; C268; C272; C274	Camaguey	Florida.CCS Saturnino Aneiro	tomato
CA282;CA288;CA294; CA297;CA299	Ciego de Ávila	Empresa de cítricos. Ceballo	tomato
SS304; SS307; SS310; SS313	S. Spiritus	Banao. ECV finca 05	tomato
SS320; SS328; SS332; SS509; SS510	S. Spiritus	Banao Flor del campo	beans
SS335; SS340; SS344; SS347	S. Spiritus	Banao Flor del campo	cucumber
VC356; VC360; VC366	Villa Clara	Caibarién. CCS Alberto Pis. C Prot.	tomato
VC381; VC382	Villa Clara	Caibarién. CCS Alberto Pis. CProt.	Pepper
VC390; VC397; VC400	Villa Clara	Caibarién. Dolores.	tomato
VC406; VC411; VC416	Villa Clara	Santa Clara. Las Marianas Huerto	tomato
VC419	Villa Clara	Santa Clara. Las Marianas. C Prot.	cucumber
VC437	Villa Clara	Santa Clara. Las Marianas. C Prot.	tomato

General molecular methods used.

Cloning: the PCR fragment from plant and *B. tabaci* (DNA 680), with taxonomic important, were purificated (Witzard PCR, Promega) and cloned in easy T vector kit (Promega, S.A). The analysis of recombinant cell was made according Sambrook et al 1988).
Sequence: The clone will be sequenced using ABI PRISM Dye terminator System (Perkin Elmer). Other PCR fragments from mtCOI gene were sequenced using these methods. The phylogeny comparison was determinate using informatics tools as Chromas, Clustal X and Easy tree 1.31 programs.
The sequences were introduced in GeneBank database (http://www.ncbi.nlm.nih.gov)

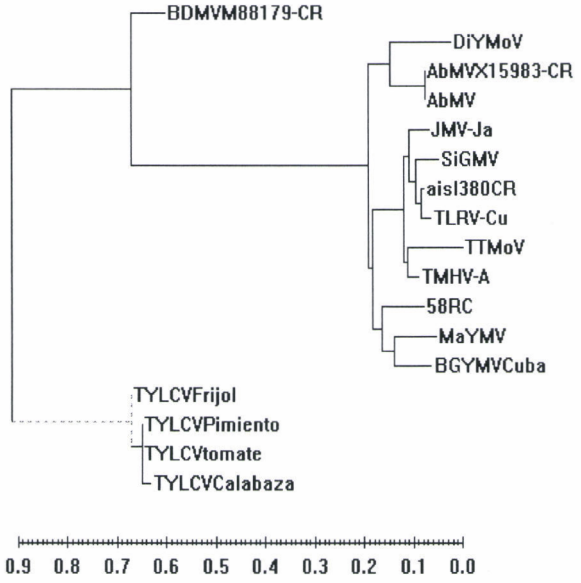
3. Results achieved

T 01.02 and T 01.06. Identification and characterization of begomovirus.

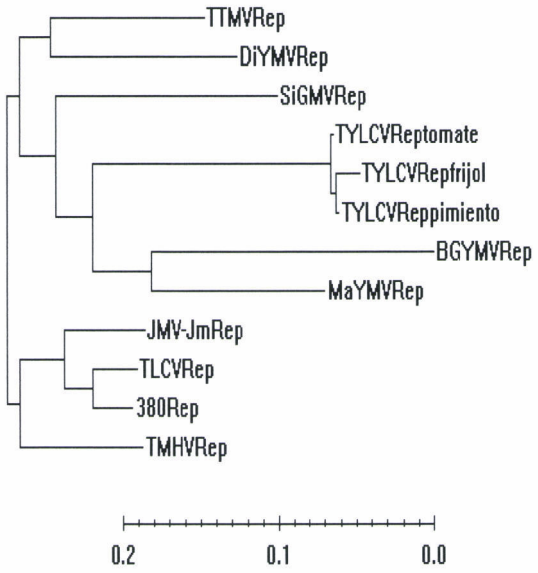
The DNA from *Jatropha gossipifolia* and *Pseudelephatopus spicatus* showed amplification products (to see annexe) with fragments of 1.5kb and 600pb for genome A and B primers, respectively. Amplification of 700pb with the specific primers of intergenic region TYLCV was obtained from *Curcubita pepo* (Squash isolate). The DNA from *Jatropha gossipifolia*, *Pseudelephatopus spicatus*, *Curcubita pepo* and other from tobacco and pepper showed PCR amplification of 600pb, with core coat protein primers.

For the impossibility of amplifying with Rojas primers (Rojas et al 1993) of genome A in tobacco and pepper plants went necessary to appeal to other areas of the genome, as core coat protein that has been referred as an sequence the taxonomic importance for the presence of sequences conserve unique that help in the identification of begomovirus not report before (Brown 2001)

The intergenic region sequence from squash (GeneBank DQ207810) showed 97% of identity with TYLCV-Isr, previously identified in Cuba in tomato, pepper and bean (Martínez et al, 1996; Quiñones et al, 2000; Martínez et al, 2002) (Figure I.1). This result is very important for epidemiological implications because *Curcubita pepo* (Squash crops) of economic interest for nutritional value is affected by the species TYLCV-Israel. The distribution, incidence and damages not yet have been determined; however for the aggressiveness of this pathogen and the sensibility of these crops to the infection for *Bemisia tabaci*, suggest strict protection measure because this pathogen can end up causing severe damages.



A



B

Figurel.1 Rooted neighbour joining tree showing the genetic distance from genome A obtained using Rojas primers (A: commune region; B: N-Terminal Rep gene)

The cloning and sequence of genome A fragment from *Jatropha gossipifolia* (designated as isolate 380, GeneBank DQ207807) showed 95% of identity with Tobacco leaf rugose virus (TLRV) identified in Cuba by Dominguez et al 2002, so much for the common region, like for the N-terminal end of the gene Rep; in these same regions it showed among 91% of identity with Jatropha mosaic virus identified in Jamaica (Roye et al., 2000) (I.1 figures A and B).
The clone from the core coat protein of *Pseudelephatopus spicatus* (isolation 58, GeneBank DQ207809) showed 98% of identity with Macroptilium yellow mosaic virus identified by Echemendia et al 2003 (I.2 figures). We can say that a *Pseudelephatopus spicatus* weed is an alternative host for MaYMV.

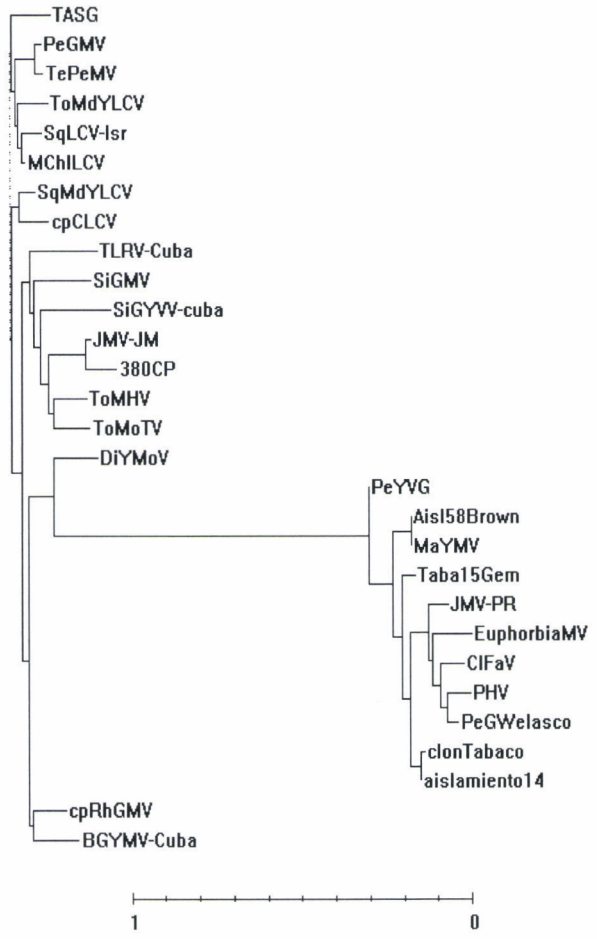


Figure I.2: Phylogeny analyze for core coat protein region sequence .

The figure I.2 shows the cluster for tobacco (isolation Tobacco) and pepper (isolation 14, GeneBank DQ207808) they have an identity of 94% with CLCV (Abouzid et al 1992b) and 98% among them. This isolates are inside the bipartite begomoviruses cluster identified in pepper in the western hemisphere, however, they separate in, different phylogeny group from the rest of the species of begomovirus identified in our country. This suggests the evidence of a new bipartite begomovirus infecting pepper and tobacco in Cuba and it constitutes the first report of the presence of a bipartite begomovirus infected pepper crops naturally in our country, however with the objective of being able to establish the necessary confirmation of serious species to conclude the sequence of the complete genome just as it has been recommended by Fauquet et al 2003. For tobacco this result proves the evidence of the presence of a new begomovirus different to the TLRV (Domínguez et al, 2002)

According the taxonomic importance of the genome regions cloned and sequenced for all the isolations, we can say that these results are an evidence of the genetic diversity of begomovirus in Cuba and the possibility that same isolations are in different host, still to know and that they play an important paper in the epidemiology of these viruses.

The detection of possible recombination events for $p \leq 0.05$ in the common region sequence can be seen in Table I.3, based on 10000 permutations. In intergenic region from 14 to 23 statistically significant fragments (Globar inner, GI) were detected between isolate58 -TMoHV; isolate58-AbMV, BDMV-MaYMV. All sites obtained in this isolates could be relationship to definitions of species for begomoviruses identified.

The probability of recombination between sequences from the common region coincides with previous reports for this region between begomoviruses from the New and Old World, from comparisons of

complete genome sequences of different genus of Geminiviridae family (Padidam et al, 1999). The common region is a place in the genome of the begomovirus where happen the biggest recombination(Padidam 1999). Our results could indicate that we are in presence of isolated recombinants, (Navas-Castillo et al, 2003; Pita et al, 2001).

The recombination is not a rare phenomenon among the begomoviruses, where additional variation factors produce prospective effects on virus pathogenesis, contributing to their evolution (Zhou et al, 1997; Harrison and Robinson, 1999; Padidam et al, 1999; Sanz et al, 1999 and Pita et al, 2001). The knowledge of sequences variants of begomovirus present in a certain geographical region, it is essential for the correct establishment management programs.

Table.3. Analysis of Recombination probability in the sequences obtained. GI. "Global inner" COMMON REGION

Fragment detected	Acronyms	P values	Number of the Polymorphism sites
GI	BDMVM88179-CR;58RC	0.0109	23
GI	AbMV;TTMoV	0.0135	15
GI	AbMVX15983-CR;TTMoV	0.0237	14
GI	TMHV-A;58RC	0.0486	21

T 01.03 Identification of *B. tabaci* biotype

As a result of the molecular analysis using the microsatellite marker Bem23, an amplification product of approximately 224 bp was resolved on an agorose gel with all the Cuban individuals except 2 that for which no amplification product was detected. The 224bp product was also detected with biotype B used as control and differed from that obtained for the biotype Q of 400bp and from the biotype A, which does not amplify this locus (Fig 1). These results suggest that the individuals analyzed except the two that did not amplify, correspond to the biotype B of *B. tabaci* and confirm the usefulness of this microsatellite marker for the differentiation of the biotypes circulating in the field. In the case of the marker MS145 some unspecific bands have rendered difficult sample identification.

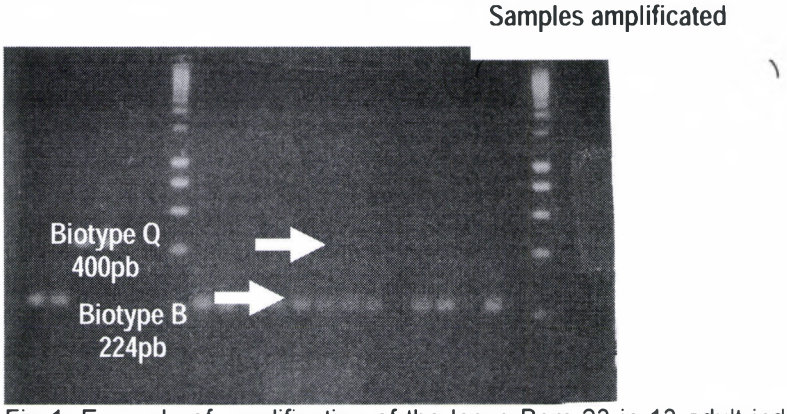


Fig 1: Example of amplification of the locus Bem 23 in 13 adult individuals of B. tabaci. Lane 7 and 22: size marker SL (Eurogentec S.A); lanes 5 and 6: Biotype A (no amplification) and lane 21: negative control.

COI gene sequencing and phylogeny analyzes.

The dendrogram obtained from the analysis of the mtCOI gene sequences (Fig. 2) showed that all the individuals from Cuba for which mtCOI sequence was obtained clustered with biotype B individuals for which mtCOI sequences were available in Gene bank. As expected, individuals taken for Q and A control clustered in two other groups.

As biotype B was shown to have originated in the Old World (De Barro et al. 2000 and Frohlich et al 1999), biotype B populations detected in Cuba have to be considered as invading populations. Biotype B populations were previously identified from Cuba together with biotype A. populations. It is supposed that, due to biological differences between biotype B and A (Bethke et al. 1991, Byrne and Miller 1990), biotype B has displaced the indigenous population as described elsewhere in Brazil and Puerto Rico (references??). It is also possible, that Biotype A was not detected in our samples because the host species on which whiteflies were sampled are not the preferred hosts of biotype A.

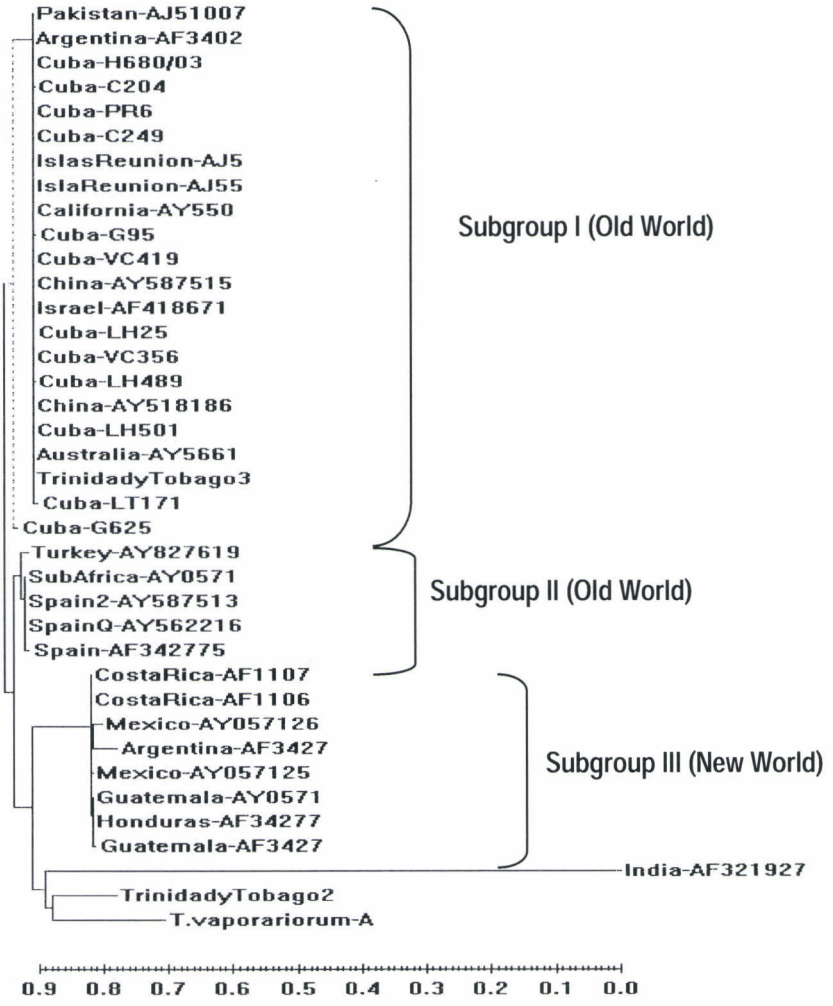


Fig. 2. Dendrogram of sequence of mtCOI gene from 14 individual of *B. tabaci* from Cuba and others different geographical regions.

These results were similar to those described by Brown and Tower-Jerez, 1999 that showed the sequence of gene mtCOI as region for separate biotype of whiteflies and to establish divergences among the individuals of *B. tabaci* biotype B, present in different geographical regions.

We were not found genetic divergences in the individuals analyzed from different localities and crops, at the present we can predict the possibility of homogeneous populations of this vector in the agricultural production fields. The effect of the TYLCV-Isr severity in the country, as well as their wide dissemination and displacement to native begomovirus can be associated with the high genetic similarity found in the individuals of *B. tabaci* analyzed in Cuba, and the present in the east hemispheres, where the TYLCV-Israel prevails and displacement in some countries the native begomovirus. These results will be an evidence or explanation to the dissemination the TYLCV-Isr native from old world in the western hemisphere and particularly in Cuba.

According to biological and taxonomic studies, Vázquez in 1995 informed the presence of the biotype B together with the A in our plantations, on a high hospedantes number in those that the wild plants are included, the results expressed in this project showed for technical molecular the existence in Cuba of the biotype B coming from the east hemiphere and indicate that this biotype has invaded and displaced the native population of biotype A. The same results have been notified in countries as Brazil, where the biotype B was disseminated by all the areas analyzed in those that previously the indigenous populations prevailed (Lima et al 2000), in a same way it happened in populations of *B. tabaci* in Israel (Horowitz et

al. 2003), in Puerto Rico (personal communication, 2002), among others. Another reason could be that the biotype A can be present in small populations that not found in the survey.

The wide range of *B. tabaci* host not analyzed in this project, as well as the appearance of new biotypes (Delatte et al , 2005) that also produce phototoxic in Squash and the genetic differences found in this biotype B, suggest the necessity to continue the molecular studies of this vector and to know on the effects associated to evolutionary processes, displacement of begomovirus races and other phenomenon that have been informed inside epidemic studies carried out with the complex whiteflies-geminivirus (Power, 2000; Zang et al 2005) and informed previously in our country.

T01.04 Development of tools for diagnosis.
We prove the primers Cu1, Cu2, Cu3 and Cu4, in combination with the primer of Rojas et at, 1993 (PAR1c496). We use the following program:

Denaturalization	94oC	5 min	
Denaturalization	94oC	1 min	30 cycles
Annealing	50oC	2 min	
Extension	72oC	1 min	
Denaturalization	94oC	1 min	1 cycles
Annealing	50oC	2 min	
Extension	72oC	7 min	

DNA of plants infected with begomovirus, positive for PCR and NAH, were selected. The best combinations were PAR1c496/Cu1 and PAR1c496/Cu3 that amplified one fragment of 1Kb. The combination PAR1c496/Cu1 amplified the tomato DNA infected with TYLCV and the PAR1c496/Cu3 it amplified with DNA from bean plant infested with BGYMV and DNA from tomato infected with TYLCV. We are analyzed other DNA from different plants that have given positive to begomovirus using Brown's primers and amplification was not obtained. The positive samples and negatives results coincided with those obtained using Rojas's primers (PAL1v1978 and PAR1c496).
The amplification was carried out tests going down the annealing temperature to 48oC and the results were same.

Two TYLCV-Isr DNA, one DNA from isolate 58 (MaYMV) and one DNA from BGYM, were amplified also using the primers sets Cu3/rep290v. We were testing two different annealing temperatures (50 and 55 oC). The results showed that amplified fragments of approximately 1.3 Kb results from both DNA of TYLCV. Besides, the two isolates of bipartite begomoviruses (MaMYV and BGYMV) amplified a fragment of some size around 1.9-2.0 Kb (Figure 3). These results implicate a need for an increase in the number of samples and the validation of these primers with DNA from different begomoviruses species.

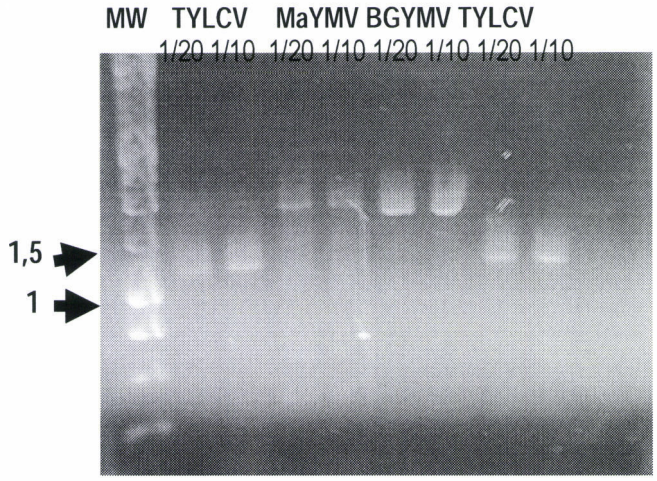


Figure 3. PCR fragment obtained from amplication using Cu3/rep290v primers

T 01.05 Distribution and incidence of begomoviruses
From 737 analyzed plants, 60.9% of the total of analyzed plants were infected with TYLCV-Isr. ToMHV alone and in mixed infections was found at a very low percentage. The comparison of these results with

those of previous years (95% of tomato infected with begomovirus) showed a decrease in the infection with begomovirus. The central and eastern tomato production areas showed bigger proportion of TYLCV infected plants compared to that of the western region with significant differences (Fig 4).

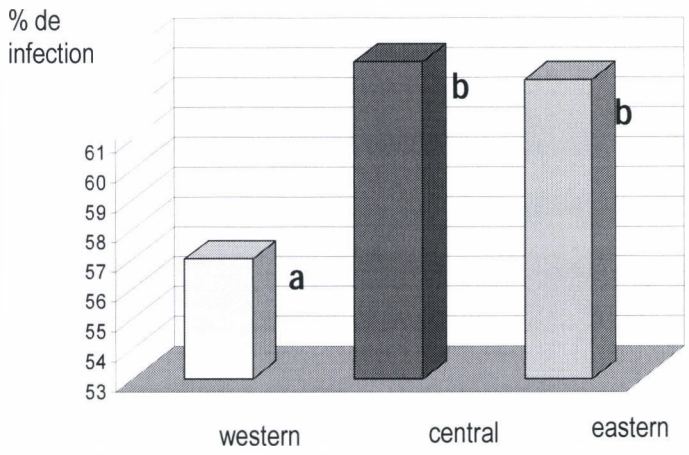


Figure 4: Incidence of TYLCV infection in different regions in Cuba.

These results indicate that the TYLCV-Isr stayed stable in the Cuban agro ecosystems. The environmental conditions have been favourable for its maintenance and its propagation towards new areas and into new varieties. The installation of TYLCV in the tomato fields in Cuba and the diminution of the prevalence of ToMHV is a phenomena which has also been observed in Florida where TYLCV is displacing ToMoV and in Spain where TYLCV-Isr. is displacing TYLCV-Sar, (Noris et al, 1994; Navas-Castillo, 1999)

Conclusions

The TYLCV is the predominant begomovirus and responsible for the infections found in the cultivation of the tomato in the country.

The TYLCV-Isr is the main begomovirus that infects tomato crop in Cuba.

The begomovirus presence was demonstrated in plants overgrowths that act as virus reservoir.

We could show the presence of begomovirus in weeds as host for viruses.

The results are the first informing on the Asteraceae family MaYMV host with recombination sites in common region with ToMHV and MaYMV previously identified in Cuba.

Presence of Jatropha mosaic virus (JMV) infecting Jatropha gossipifoli in Cuba.

First report of bipartite begomovirus in pepper crop in Cuba.

First report of TYLCV-Isr in infecting naturally squash crop.

We could show wide begomoviruses diversity in Cuban agroecosytems.

The whiteflies individuals characterized in different localities and host were biotype B from east hemisphere.

T 01.07 Transmission bio test of TYLCV by *Bemisia tabaci*

In standardized conditions the acquisition access period and inoculation access periods was 48 hours and were used 3, 10, and 25 infective females on single tomatoes plants at the 2 true leaves stage. We can determine the transmission rates for TYLCV from Cuba from tomatoes to tomatoes (figure 4) and from tomatoes to pepper (table I.4):

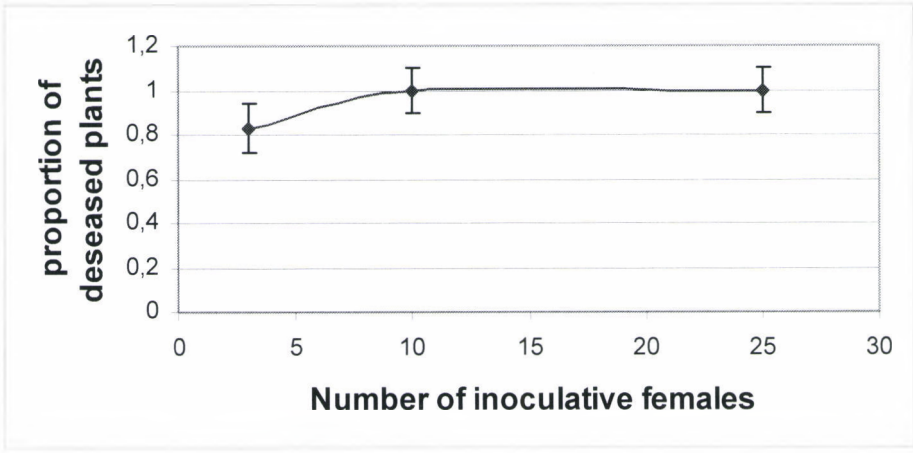


Figure 5. Transmission of TYLCV to tomato by females of *Bemisia tabaci* biotype B with 48h AAP and 48h IAP. Vertical bars correspond to 95% confidence interval

The transmission of TYLCV obtained was similar to that of other persistent viruses. Brown and Nelson (1988) determined that the relative efficiencies of virus transmission by 1, 5, 10 and 20 whiteflies a 3 days IAP after a 48 hr AAP were 84% and 100% with 10 and 20 whiteflies respectively. Pico et al (1996) observed for 10 to 20 viruliferous whiteflies efficiency in the transmission of 100% for different isolates of TYLCV. For these reasons the values obtained in this study are in correspondence with that found by these authors.

Table I.4. Transmission rate of TYLCV by *Bemisia tabaci*, from of tomato to pepper

No infective female	Transmission rate (%) TYLCV
3	0
10	0
25	16

The transmission in pepper was very low and alone we were results for a level of 25 females infected to the 30 days.

The comparison of the rate of transmission of the TYLCV of tomato to tomato and of tomato to pepper shows that there was a better efficiency in the transmission to tomato for all the variants of used females, however in pepper was very low for whiteflies level and times analyzed. These results suggest that different factors could be affect the efficiency of transmission or multiplication of the virus in this crop and they can explain the low level of distribution and aggressiveness of the TYLCV found in the pepper crops under natural conditions. The high infection levels for TYLCV in tomato crop evidences that even when the infection levels are low is necessary the implementation of management because since 3 whiteflies the transmission rate rises above 80%.

WP B. EPIDEMIOLOGY

Taks 02.03 y T 02.04.

During the 2002-2003 and the 2003-2004 seasons two experimental fields were planted, 0.25ha each one, which consisted of a begomovirus infection susceptible variant and of HC3019 hybrid (Israel), tolerant to the disease and used in the production. Both cultivars were planted and taking into account all the measures having shown on the survey for the three regions an influence on the severity and incidence of the disease.

Possible weather factors that might relate to disease development were measured daily in a meteorological located at the experimental area, with an equipment properly validated and calibrated by the department of metrology and quality control of the Nacional Institute for Meteorology in Casa Blanca.

Weather factors analyzed were the following:
Wind (direction and speed)

Air temperature (dry average, Tmax., Tmin., Average Tmax-Tmin, Oscilation)
Relative humidity (maximum, minimum and average).
Precipitations
Insolation and Isolation percent

Weekly samplings were done starting 15 days after transplant; adult counting in field, incidence of symptoms as well as sample collection were practiced in order to analyze the presence of the virus through laboratory diagnosis based on non-radioactive nucleic acid hybridization. Samplings were done diagonally, taking four points in each diagonal plus the centre (crossed diagonal). These data were grouped into pro, against wind direction and centre field samples for further analyses.

STATISTICAL ANALYSES:
A main component analysis was carried out so as to determine the influence of weather factors having a greater impact on the incidence and severity of the virus in the field using the SPSS software (version 5.0 for Windows) (Visauta, 1998).

DISPERSION INDEX AND APPLICATION OF MODIFIED TAYLOR`S LAW
To assess disease dispersion, those fields planted with Amalia variety were chosen, which were divided into 5 rectangular boxes; individual plants were assessed in terms of presence or absence of symptoms. The proportion of infected plants was determined for each quadrant (p), using the equation: $P=\Sigma Xi/nN$

Where n is the number of plants in each boxes, N the total number of quadrants per field and ΣXi is the summary of the number of plants infected in each quadrant i.e These data were used to calculate the dispersion index.

To evaluate the fields and the whole of fields sampled, the observed variance (Vobs) and the expected binomial variance (Vbin) were calculated by using the equations:
$Vobs=\Sigma (Xi-np)2/n2(N-1)$
$Vbin=p(1-p)/n$
$ID=Vbin/Vobs$.

Modified Taylor`s law was used according to Batista, 2001. The regression equation and parameters A and b were calculated as randomness indicatives according to the STATISTICA 8.0. Software.

PREDICTION ANALYSES
In order to predict the behaviour of the infection from weather conditions and taking into account control measures in all experiment, quadratic regression analyses as well as a surface response representation were done both for the density and the infection percent, using the STATISTICA 8.0 sofware.

RESULTS AND DISCUSSION
ANALYSIS OF WEATHER FACTORS THAT INFLUENCE IN DISEASES.
Table III.1 shows the main component analysis in which it was demonstrated that changes and temperature, relative humidity and average insolation are the weather parameters explaining between 50-70% the variability of the whitefly-begomovirus complex under the conditions assessed.
Table III.1 Main parameters explaining the variability found in field.

	CP1	CP2	CP3	CP4	CP5
Vel.Viento	0.186589	0.206351	-.509231	0.373596	-.012593
Temp. Media	0.427513	0.101237	-.013359	-.134716	-.148376
Temp. Máx.	0.419927	-.114352	-.036658	-.105152	-.299815
Temp. Mín.	0.413275	0.180516	-.002125	-.013518	-.162218
Temp. Prom.	0.434433	0.046474	-.014678	-.064894	-.236956
Oscilación	-.035043	-.514812	-.059013	-.145237	-.203414
HR maxima	0.095205	0.055509	0.715422	0.368230	-.018200
HR minima	0.041199	0.414147	-.107387	-.563463	0.462738
HR promedio	0.170811	0.354767	0.383309	-.060065	0.134127
Precipit.	-.109849	0.392251	-.233220	0.504321	-.065326
Insolación	0.324220	-.321327	-.103827	0.081464	0.380890
Insol.Prom.	0.296983	.277029	0.000646	0.296757	0.618308
Var.expl.	0.4201	0.2795	0.1292	0.0675	0.0485
Var. acum.	0.4201	0.6997	0.8289	0.8953	0.9449

It is known that weather components are denso-dependent factors producing changes on population density of insects. Specifically, temperature and humidity determine the optimal conditions for the development of these individuals. This is due to the poichilothermic behaviour of many individuals as well as the characteristics of these weather factors that vary in a higher proportion from one day to the next. This was shown in this experiment where out of 12 weather components assessed, temperatures rank as the first factor in order of importance to explain other processes. However, in order to explain more than 90% of variations, there is a need to include all the factors studied. In fact, all of them may influence population changes.

Table III.2 shows that in both varieties was a correlation between the percent of infected plants, the density of whiteflies, the incidence and severity of symptoms. Although the correlation was positive, it was not found a high significance in the relation percent of infection-severity of symptoms for the 30-19 variety, likely as a result of the low percent of infection. Nonetheless, even if the relation infection-incidence is not very high (0.6255), it turned out to be significative (p=0.022). For the Amalia variety, the correlations of the infection percent and the density of whiteflies, the severity and the incidence resulted significant with values of 0.5417, 0.8020 and 0.9105, respectively.

Table III.2. Correlation between the infection percent and whiteflies density, the incidence and severity of disease.

Factors		Variety 30-19	Amalia
Densidad of whiteflies	r	0.1660	0.5417
	p	0.587	0.055
Incidence	r	0.6255	0.9105
	p	0.022	<0.0001
Severity	r	0.2566	0.8020
	p	0.397	0.001

When comparing the behaviour of the two varieties studied, it was observed through the simple variance analysis (table III.3 and Figure III.3) that the incident, severity and percent of infected plants was significantly higher ($p<0.05$) in the susceptible variety than in the tolerant one, kept both under good environment conditions, good cultivation practices and no significant differences regarding whitefly density. The results corresponding to severity and incidence according to symptoms observed were corroborated by molecular diagnosis, specifically through non-radioactive nucleic acid hybridization. This confirmed that infection percents derived from hybrid HC 3019 were significantly lower than those obtained for the Amaila variety. (Figure III.4). These data confirm the importance of introducing tolerant or resistant varieties in IPM programs and confirm those obtained in surveys, where the susceptibility of the variety has a greater significance on the incidence percents and the severity of infection.

Table III.3. Behaviour of varieties

Variety	Density of whiteflies	Incidence	Severity	% Infection
Aro30-19	1.73 ± 0.16 a	0.06 ± 0.04 b	0.04 ± 0.02 b	18.71 ± 8.88 b
Amalia	1.62 ± 0.15 a	0.29 ± 0.08 a	0.24 ± 0.08 a	59.37 ± 9.89 a

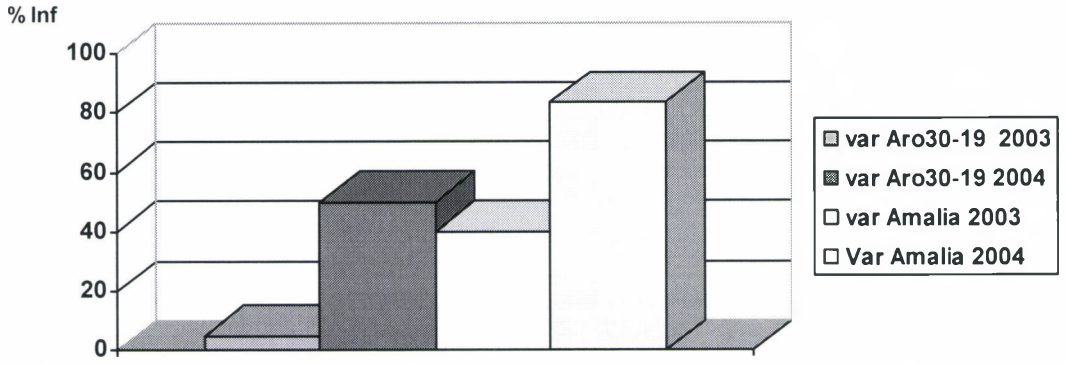


Figure III.3 Percent of plants infected in each of the varieties assessed for the two-year period analyzed.

In general, both the density of the whitefly and the severity, incident were lightly higher in 2004 (figures III.4 and III.5). However, the percent of infected plants in 2003 corresponding to variety 30-19 was extremely low (4.81) and reached 50% in 2004, for the Amalia variety there was also an increase from 39.91% in 2003 to a 83.67% in 2004.

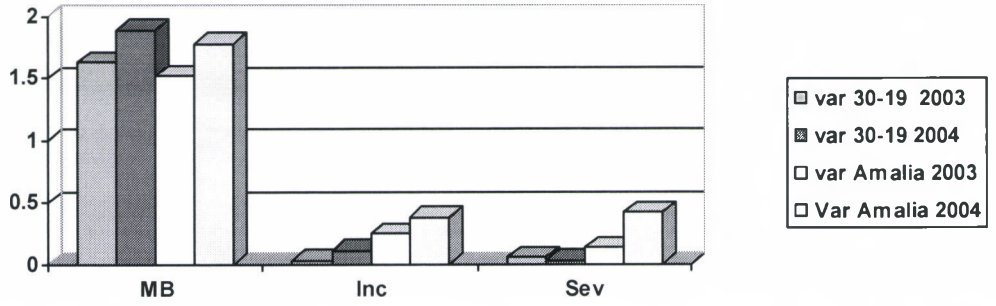


Figure III.4 Analysis of the density of whiteflies, incidence and severity over the two-year period for both varieties.

In both years, it was observed that the incidence and the severity of the infection appears for the first time at 6 weeks after the first survey for the Amalia variety (approximately at 60 days after transplantation) anda t week 8 for the Aro 3019 hybrid (approximately 75 days after transplantation). Even if infection occurred in the field for both varieties, the highest impact was attained after blooming of the crop, which decreases the repercussion of infection on yield and productivity levels.

Dispersion index

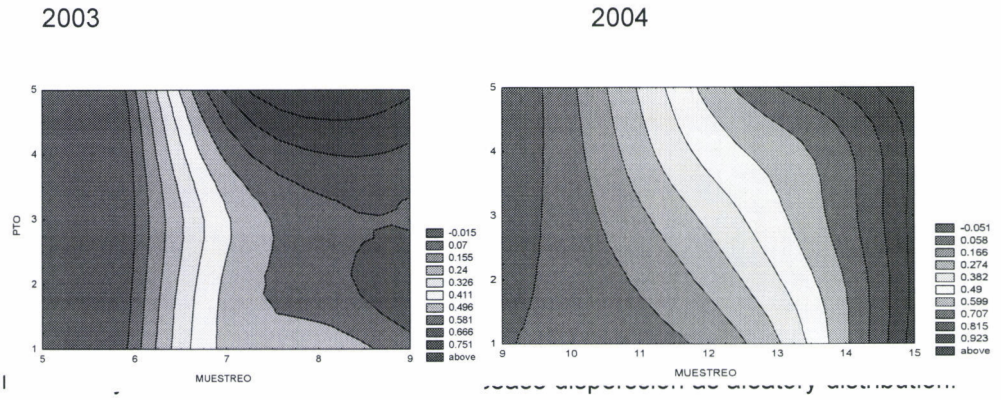
The disease dispersion index was determined in each year analyzed in Amalia cultivar, as see in table III.4, the probability of infection for plant in both years, was always between 34 and 59%.

Table III.4 Diseases Dispersion index in Amalia cultivar in each year studied ($P<0.05$)

Year	Survey number	Probability of infection for plant	Dispersion index (ID)
1	1	0.59	0.36651515
1	2	0.52	0.25865285
1	3	0.66	0.44
2	4	0.34	0.17463035
2	5	0.47	0.14960961
2	6	0.59	0.1025

Using regression mean analysis a b=0032 value was obtained for a $P < 0.05$, where $b < 1$ is a aleatoridad indicator, this result it was confirmed later when an dispersion index was obtained in all the

cases minor that 1. This result confirms that the disease distribution in the field is Aleatory, as show the figure III.5.



Figui

Prediction develops of severity and incidence of disease index and whiteflies density.
We could be predict that whiteflies density using quadratic curved that denote a local minimum, with increase to the highest temperatures obtained inside the season (III.6 Figures A and C). The space of phases shows optimal values (of bigger density of the whiteflies) for temperatures among 24 - 25°C and relative humidity among 78-84%. On the other hand, the biggest infection percent was detected with temperatures between 22 and 24°C and inferior humidity to 74% (III.6 Figures B and D)

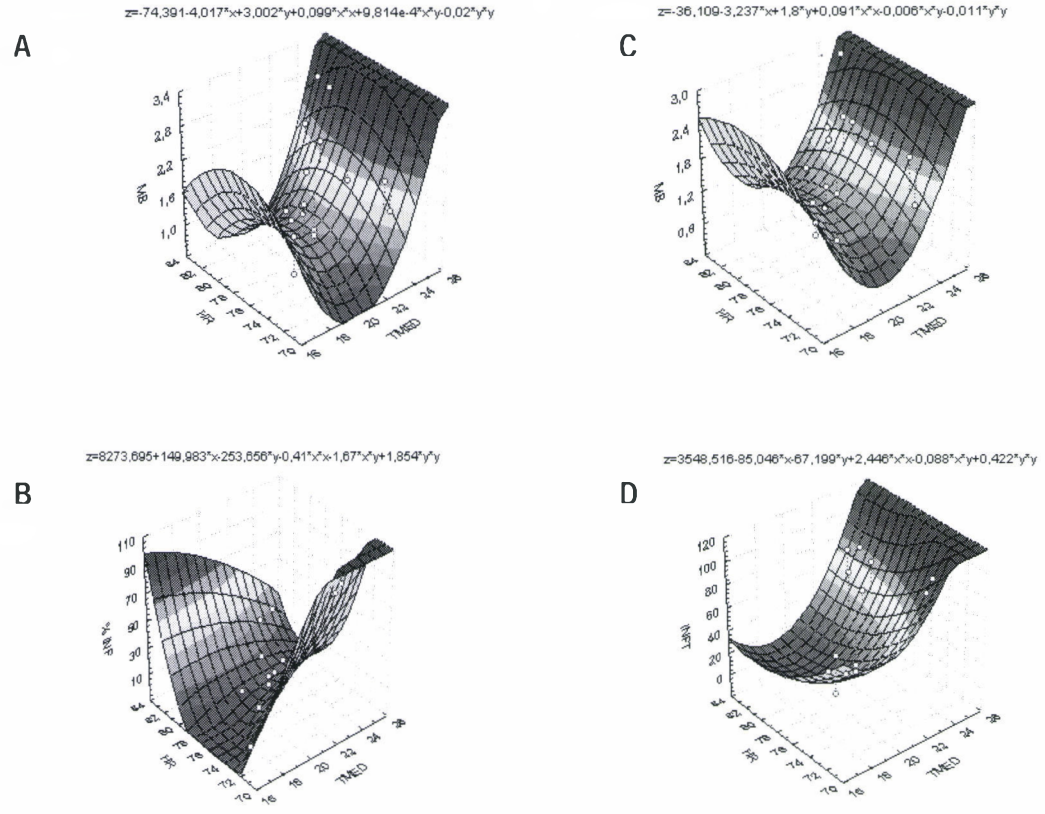


Figure III.6 Prediction studies for whiteflies density and incidence and severity of disease with temperature and humidity changes. A and D) Prediction of whiteflies density in Aro 3019 and Amalia cultivar, respectively; B and C) Prediction of infection in Aro 3019 and Amalia cultivar, respectively.

In conclusion, the relative humidity and temperature had significant influence in whiteflies density, incidence and severity disease.
The TYLCV-Isr infections in fields have the aleatory distribution index.

4. Problems encountered
None

5. Technology implementation plan
Not applicable

6. Publications and papers
Papers in international journal,

Yamila. Martínez, Damian Fonseca, Iris Palenzuela and M. Quiñones.(2004) Presence of Tomato yellow leaf curl in squash (Curcubita pepo) in Cuba. Plant Disease vol. 88 No.5 page 572.
O. Gómez, M. Piñon, Y. Martinez, M. Quiñones, D. Fonseca and Laterrot (2004) Breeding for resistence to begomovirus in tropic-adapted tomato genotypes Plant Breeding volume 123: Issue 3, 275-279.
Y. Martinez Zubiaur, Y. Muñiz and M. Quiñones Pantoja. First report of a bipartite begomovirus infecting pepper plants in Cuba. Plant Pathology Journal, NRD-2006-35, in press

Papers in national journal.

Yamila Martínez, Madelaine Quiñones y Damian Fonseca. (2003) National Survey of tomato begomovirus in Cuba. Rev. Protección. Veg. Vol.18(3)168-175.
M. Quiñones, D. Fonseca, O. Gómez, Ileana Miranda, M. Piñón and Yamila Martínez(2003) Optimisation and application of the non-radioactive nucleic acid hibridization for the diagnostic of Tomato Yellow Leaf Curl Virus (TYLCV) in the breeding program. Rev. Protección. Veg. Vol.18(3)176-182.
Madelaine Quiñónez, D. Fonseca y Y. Martínez. (2004)Comparación de métodos de hibridación de ácidos nucleicos para el diagnostico del virus del encrespamiento amarillo de la hoja del tomate (TYLCV) en Cuba. Rev. Protección Veg. Vol 19 (1): 26-32.
Madelaine Quiñonez (2004).Distribution, genetic variability and optimization of Tomato yellow leaf curl virus (TYLCV) diagnostic in Cuba. Rev. Protección Veg Vol. 19(1):72

PhD in Agricultura Science Thesis Distribución, variabilidad genética y perfeccionamiento del sistema de diagnostico molecular del virus del encrespamiento amarillo de la hoja del tomate (TYLCV) en Cuba.

National and International Workshop

- 43th Meeting of America Phytopathology Society, Caribbean Division, Antigua, Guatemala, Junio, 2002
- 44th Meeting of America Phytopathology Society, Caribbean Division, Texas, Estados Unidos, Abril, 2003
- Taller Internacional de Ordenamiento y Desarrollo Rural, Habana, 2003
- 45th Meeting of America Phytopathology Society, Caribbean Division, Habana, 2004
- V Seminario Internacional de Sanidad Vegetal, Habana, 2004
- II Encuentro Internacional de Vigilancia Fitosanitaria, Habana 2005

7. Conclusion
This project strengthens the knowledge of the begomovirus biodiversity in Cuba and permitted the molecular characterization of the biotype B of *B. tabaci* as more predominance populations in tomato and other crops and weeds adjacent. These results together to the epidemiological studies, we could to have a starting point and scientific support, for the improvement the management integrate for tomato crops in our country.
These results will be to know aspects that should go until the specifications for localities or agro ecosystems for tomato production and rigorous measures with the definitions for management in general.

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ANNEXE

Resultados obtenidos de las amplificaciones con los cebadores genericos y específicos utilizados para la identificación y diagnostico de los begomovirus en las plantas especies.

Familia	Especies	Inic. Gen.		Inic. Esp.	Cebadores gen. de la región central de CP
		A	B	TYLCV	
Euphorbiaceae	Euphorbia heterophyla	+	-	+	+
	Jatropha gossipifolia	+	+		+
	Caperonia palustris	-	-	-	-
	Rhyncosia sp	+	-	-	+
Convolvulácea	Ipoema sp.	-	-	-	-
	Marremia umbellata	-	-	-	-
Curcubitaceae	Cucumis augunia	-	-	-	
	Curcubita pepo	-	-	+	+
	Mamordica charantia	-	-	-	
Asteraceae	Malanthera deltoidea	-	-	-	
	Pseudelephatopus spicatus	+	+	-	+
Malváceas	Sida acuminata	-	-	-	-
	Malvastrum coromandelianum	+	-	-	-
	Sida procumbus	-	-	-	-
	Sida glomerata	-	-	-	-

	Sida rhobifolia	-	-	-	-
	Malachra ureus Poit	-	-	-	-
	Sida acuta	-	-	-	-
Verbenáceas	Phyla rodiflora	-	-	-	-
Commelinaceae	Commelina sp	-	-	-	-
Boraginaceae	Heliotropium indicum	-	-	-	-
Sterculinaceae	Walteria indica L.	+	-	-	-
Amaranthaceae	Amaranthus dubius	-	-	-	-

Begomovirus presentes en Cuba, utilizados en los análisis filogenéticos

Virus	Acrónimo	Número de Accesión	Autor
Bean golden yellow mosaic virus	BGYMV	AJ544531	Echemendia et al, 2003
Tomato mottle Taino virus	ToMTV	NC001828	Ramos et al, 1997
Tomato Mosaic Habana virus	ToMHV		Martinez et al, 1998
Macroptilium yellow mosaic virus	MaYMV	NC004731	Ramos et al, 1999
Dicliptera yellow mottle virus	DiYMV	AJ549960	Echemendia et al, 2003
Tobacco leaf rugose virus	TLRV	TLE488768	Dominguez, M et al, 2002
Sida golden yellow vein virus	SiGYVV	AJ577395	Echemendia et al, 2003

INDIVIDUAL PARTNER P7 FINAL REPORT

PARTNER 7: Instituto de Investigaciones Hortícolas "Liliana Dimitrova", (IIHLD), Cuba

Olimpia Gómez

1. Objectives
Tasks
T02.02, T03.01, T03.02, T03.03, T03.04

2. Activities

3. Results achieved
WPB - Epidemiology and models to investigate the appropriate mix of disease control tools at both crop and ecosystem levels
The aims of this work package are (i) to identify the factors (biotic and abiotic) that are key components of begomovirus epidemics in the Caribbean islands and (ii) to develop a model to facilitate the designs of IPM packages.

T02.02 Target survey to validate the agrosystem model
An epidemiological survey was carried out in Cuba in order to identify the key factors of begomovirus epidemics. The IIHLD developed the survey at the western part of the Island including from Pinar del Río to Ciego de Ávila provinces. Data were offered to partner P2 in order to be processed and analized in the ecosystem model construction.

WPC - Evaluation of IPM packages on crop management and resistant varieties
The aims of this work package are: (i) to define different strategies of virus control based on the use of tolerant/resistant cultivars and the appropriate crop management according to WPB results, and (ii) to IPM evaluation in different agronomic and socio-economic conditions. Finally, an integrated control system reducing the economic impact of begomoviruses in tomato in the Caribbean should be defined.
The evaluation of the tomato plants were done on two major criteria: i) resistance to the begomoviruses, ii) resistance to other main pathogens such as Ralstonia solanacearum. The adaptation of the cultivars to heat and to the fresh market (main production in the Lesser Antilles) and to process tomatoes (part of the production in the Greater Antilles) should be relevant and included into the criteria of evaluation as criteria iii) adaptation to heat and iV) adaptation to local markets.

T03.01 Screening varieties for resistance to the identified begomoviruses
Thirteen trials were carried out in the project (3 in Martinique, 3 in Trinidad and 7 in Cuba) which involved 375 entries that were tested for their resistance to the different begomoviruses reported in the participant countries (TYLCV, Cuba and Guadeloupe; PYMV, Guadeloupe and PYMTV, Trinidad) as well as for their productivity and market acceptance.

Cuba
Tomato F1 hybrids resistant to TYLCV
The best F1 hybrids that highlighted through the trials carried out in Cuba were finally tested under two environments, optimum crop season and off season, during two years. TYLCV-like symptoms weren't observed in them. Different productivity characters were studied, some of which, are showed in Table 1.

Table 1. Highlighted hybrids tested in two environments: optimum crop season (O) and off season (T)

Number (Figures)	Hybrid	Season	% Fruits highest grade (*)	Mean fruit weight (g)	Yield (Kg/ plant)
1	32	O	3	81	5,38
2	32	T	9	122	4,35
3	41	O	3	69	4,68
4	41	T	8	107	5,83
5	48	O	6	90	7,15

6	48	T	6	107	5,87
7	7	O	6	101	7,36
8	7	T	10	119	4,22
9	34	O	3	83	5,28
10	34	T	8	121	3,75
11	35	O	3	88	5,34
12	35	T	11	124	3,67
13	39	O	3	86	5,06
14	39	T	10	115	4,86
15	57	O	8	85	6,65
16	57	T	21	116	4,11
17	70	O	3	80	6,90
18	70	T	14	126	3,99
19	24	O	3	108	6,47
20	24	T	12	129	3,73
21	60	O	29	136	5,10
22	60	T	63	184	4,25
23	HA 3019	O	37	144	2,85
24	HA 3019	T	36	144	1,43
25	HA 3105	O	10	120	5,36
26	HA 3105	T	10	111	2,54

*fruit diameter>8.5 cm

The phenotypic correlations were calculated from the original data, these were useful to know some associations between the studied characters, such as:

• Yield depends on the total number of fruits per plant showed by the genotypes in both environments (r2=0.822 and 0.941, optimum crop season and off season, respectively).
• In spite of, in the studied hybrids mean fruit weight got a negative and significant correlation with the total number of fruits per plant in both environment (r2=-0.839 and -0.603, respectively), it depends positively on the number (r2=0.858 and 0.788, respectively) and weight (r2=0.900 and 0.825, respectively) of fruits from the highest grade (fruit diameter>8.5 cm).

A Principal Component Analysis made let the genotypes to be grouped (STATGRAPH Plus version 5). In this case, the first two components account for 80.011% of the variability from the original data. The number of total fruits and the mean fruit weight, both yield components, got the highest contribution in Component 1 and the number of fruits and the mean weight from 1st grade fruits got the highest contribution in Component 2 (Table 1). According to principal components (Figure 1), the genotypes could be grouped and the best choice may be the F1 hybrids that occupy the first quadrant (numbers 4, 5, 6, 7, 11 and 19 at Figure 2), corresponding in Table 1 to the F1 hybrids: 7, 24, 35 at optimum season and 41 at off season, while the F1 hybrid 48 may be recommended for both, optimum and off season.

Table 1. Component weight

Variables	Component 1	Component 2
Number of fruits of 1st grade (N1a)	0.265963	0.405809
Number of fruits of select grade	-0.318449	0.327644
Number of total fruits (NT)	0.397335	0.054856
Weight of 1st grade fruits (P1a)	0.214077	0.459475
Weight of select grade fruits	-0.334937	0.317359
Weight of total fruits	0.336512	0.313105
Yield (Rend PT)	0.346508	0.313155
Mean fruit weight 1st grade fruits	-0.225603	0.331519
Mean fruit weight select grade fruits	-0.293962	0.257781
Mean fruit weight (XT)	-0.38494	0.201666

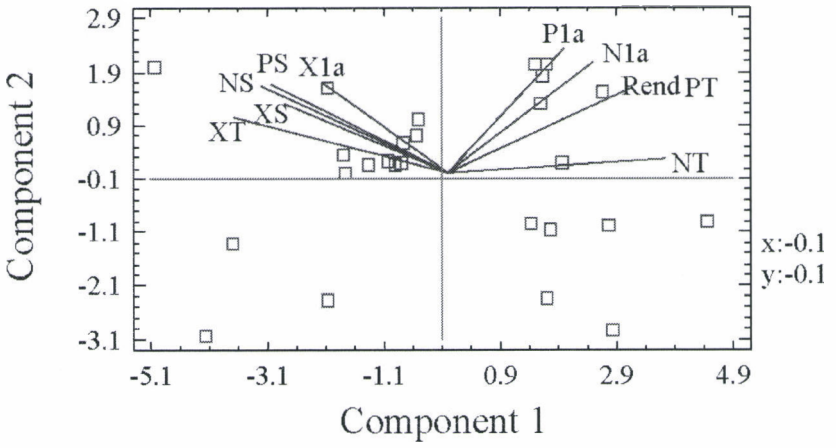


Fig. 1 Variable distribution

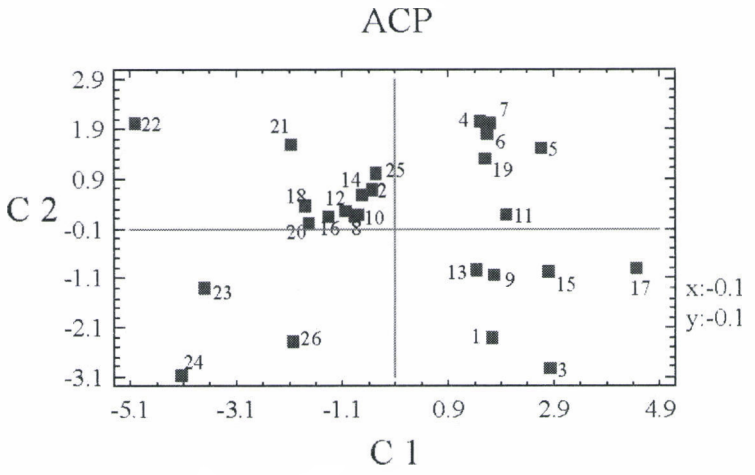


Fig. 2 Hybrid distribution

While, also from the original 26 cultivars data matrix, a Cluster Analysis procedure let to create five clusters at a short (80) Euclidian distance. The F1 hybrids: 4, 5, 6, 7 and 19 were included at group III confirming similar characters between them (Figure 3).

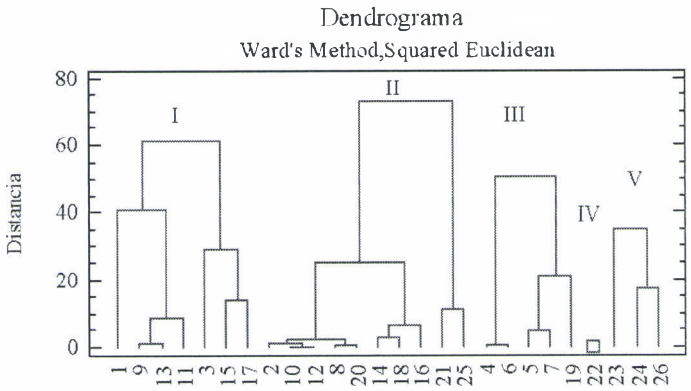


Fig.3 Hybrid cluster

The factorial ANOVA showed that the interactions between the variation sources studied: genotype x season, genotype x year and genotype x season x year were significant ($P<0.01$). The F1 hybrid 48 showed the most stabile yield according to the Principal Coordinated Analysis made.

Other tomato cultivars resistant to TYLCV

The fresh market tomato variety ´Vyta´ got a high implementation level in Cuban commercial production (10 000 ha) during the period 2002-2005, avoiding yield losses because of begomovirus and turning production independent to chemical protection. This Cuban variety was also tried by other partners. ´Vyta´ main characteristics are:

- Resistant to Tomato yellow leaf curl virus (TYLCV).
- Resistant to Fusarium oxysporum and Stemphylium spp.
- Heat and humidity tolerant.
- Determinate growth habit.
- Early (100-110 days) and productive.
- Round, medium fruits (115 g), uniform colour at ripening.
- Local market adaptation.

Resistance to different TYLCV- begomovirus isolates

- Resistance to the Cuban isolate. Four tomato lines introgressed from Lycopersicon chilense (LA 1969) were compared with commercial F1 hybrids 'ARO 8479´ and ´HA 3108´, which are tolerant to TYLCV, and cv. ´Campbell 28´ as a susceptible control. Resistance was evaluated by grafting diseased scions and in a field trial where plants infected by viruliferous whiteflies and disease-free plants (control) were transplanted.

The lines LD 3, LD 4, LD 5 and LD 6 showed no disease symptoms after grafting and in the field trial. Virus accumulation at 60 days after transplanting was low in the infected plants: 0.09, 0.60, 1.00 and 0.50 nanograms, respectively. No fruit-set and yield losses were registered under high temperature conditions prevalent in the trial, whereby lines LD 5 and LD 6 were better adapted to tropical conditions. Viral DNA concentrations were over 1 000 nanograms in cvs. ´Campbell 28´, ´ARO 8479´ and ´HA 3108´. The last two are considered tolerant as they were asymptomatic or had mild symptoms, respectively, but got acceptable yields in the trial. By contrast, virus had a negative effect on fruit-set, number of fruits per plant and total yield in cv. ´Campbell 28´ (Gómez et al, 2004).

In order to explain the field performance of these lines, a characterization by molecular markers (RFLP) was done. Twelve tomato genotypes were tested, including breeding lines as well as patterns known as resistant or susceptible to TYLCV and tolerant commercial hybrids. Plant DNA extraction, digestion, hybridizations and autoradiography were done according to the protocol used at INRA-Avignon, France. The separation of the DNA fragments was made by 0.8 % agarose gel in TAE buffer. The genomic probes TG 97 and TG 297 were used as they have been reported strongly linked to TYLCV disease plant

response. Four breeding lines (LD 3, LD 4, LD 5 and LD 6) and the patterns resistant to TYLCV (Lycopersicon chilense and Ty-52) carried the Ty-1 gene, as indicated by TG-97 marker but not by TG 297 (Figure 4). The commercial hybrid 'Fiona' carried the Ty-1 gene and a second band which can be linked to a proper variant of this genotype, common or not, with other resistant sources. The breeding line 13-8-1 and the commercial hybrid 'HA 3105' don't carry the Ty-1 gene, indicating that the genetic basis conditioning resistance/tolerance comes from another wild source but not from L. chilense. The lines Ty 50 and 13-8-2, susceptible at the field, as the control 'Campbell 28', showed the susceptible varying at the zone monitored by TG-97. These results indicate that different sources of resistance are present in the breeding lines tested which also have favorable horticultural traits which might be used in further breeding facilitating the development of pyramidizing Tomato leaf curl virus resistance genes (Piñón et al, 2005).

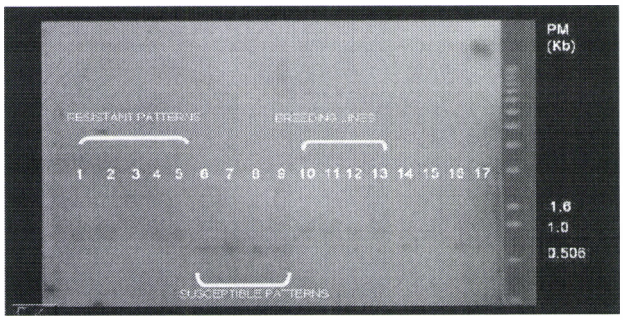


Fig. 4 Detection of Ty-1 gene introgressed from L. chilense

• Resistance to the Reunion Island isolate. Three tomato lines and a variety reported resistant to Tomato yellow leaf curl virus in Cuba were tested to TYLCV isolate from the Reunion Island (TYLCV-Mld [RE]) as well as the Israelian resistant line TY-7 and the TYLCV-susceptible lines TY-1 (Israel), 13-8-2 (Cuba) and cv. 'Farmer´. Resistance was evaluated by using viruliferous whiteflies and virus diseased scions, in these conditions, the TYLCV-resistant lines: 13-8-1, LD 5, LD 6, TY-7 and cv. 'Vyta´ didn't show viral disease incidence and symptoms in the plants. On the contrary, the TYLCV susceptible lines TY-1, 13-8-2 and cv. 'Farmer' showed TYLCV-Mld [RE]-like strong symptoms after vector inoculation and grafting (Figure 5). After grafting inoculation, virus presence (couldn't quantify) was detected in 100% of the plants of all the cultivars when testing by Heap-Elisa, kit DSMZ, FRG (Piñón and Dintinger, 2006).

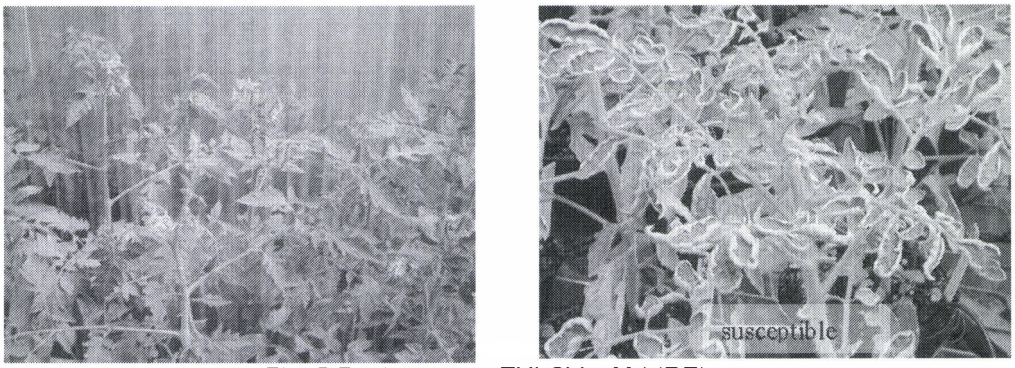


Fig. 5 Resistance to TYLCV – Md (RE)

Besides, some PCR polimorphics markers (SSR microsatelites) which could be associated to begomovirus resistance were tested in an F1 cross between sister lines, resistant and susceptible (13-8-1 X 13-8-2) belonging to the IIHLD tomato breeding program. The work must continue in the progeny in order to verify the possible isogenicity of the sister lines.

T03.02 Screening varieties to adaptation to Caribbean constraints

• Ralstonia solanacearum. To improve the output of begomovirus resistant cultivars in areas of the Caribbean Basin where bacterial wilt (Ralstonia solanacearum) is also a major constraint for tomato

(French West Indies and some places in Trinidad), some cultural practices may be adopted, such as grafting. According to the results carried out by Partner 1 in Martinique, particular considerations were established.

• Nematodes. The previous standardisation of a PCR-based test to evaluate nematode (Meloidogyne spp) resistance in tomato, using primers designed in Cuba, served to detect the Mi gene in the F1 hybrid ´LTM 12´ (former Hybrid 24 at Table 1). This test is based in a codominant marker (REX-1) tightly linked to Mi gene. The procedure is used for rapid routine screening instead of resistance test (Figure 6).

susceptible

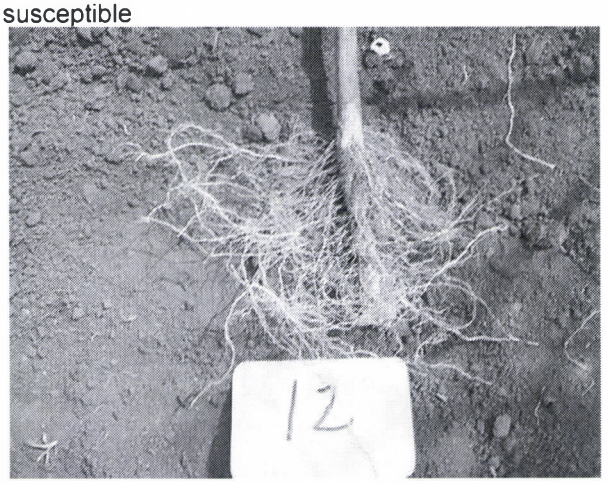


Fig. 6 Resistance to nematodes

T03.03 Workshop Wo2 and designing of IPM packages

In order to design an IPM package to be evaluated in terms of disease control and economic impact to achieve the last milestone of the work package C, a workshop was organized in Havana, Cuba, by Partners 6 and 7 during the 3rd Annual Meeting of the project (November 29-December 3, 2004). In this opportunity the agronomical practices to be considered were defined.

Two IPM plots were designed for Cuban and French West Indies conditions that were compared with traditional method plots by Partners 1 and 7. The characteristics of the trials and the evaluations needed were also defined at the workshop.

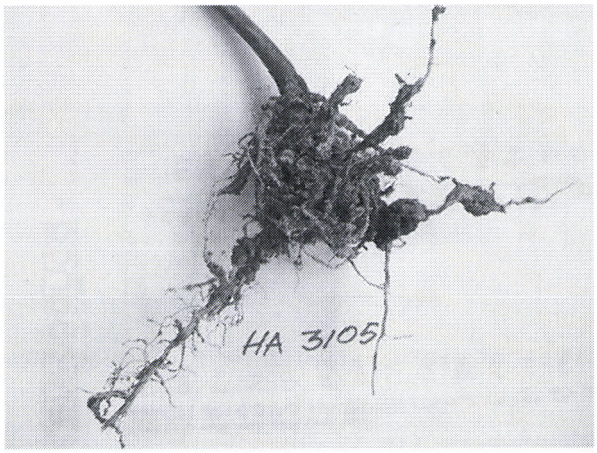
T03.04 IPM packages evaluation

For Cuba, where Tomato yellow leaf curl virus (TYLCV) has a great distribution, different factors were combined in the treatments tested:

TI - Traditional practices + susceptible variety: tomato cv. ´Amalia´ (well adapted to tropical conditions but susceptible to TYLCV); plantlets grown under classical nursery conditions (bare root, non isolated); use of Imidacloprid to vector control.
TII – Traditional practices + resistant variety: tomato cv.´Vyta´(well adapted to tropical conditions and resistant to TYLCV); plantlets grown under classical nursery conditions (bare root, non isolated); use of Imidacloprid to vector control.
TIII – IPM practices: tomato cv.´Vyta´; plantlets grown in speedlings, insectproof isolated; use of Verticillium lecannii to vector control.

Two trials were carry out at IIHLD during the dry season, which is more favourable to grow tomatoes in the Island because of low temperatures but when *B. tabaci* populations are high, so the risk of begomovirus infection is also high.

The *Bemisia tabaci* population after transplanting showed no differences between treatments (Figure 7). Nevertheless, TI got an increased severity from the second (1.51) to the seventh week (3.72) after transplanting which meant severe damages in plants, while in TII and TIII severity kept below 0.6 (Figure 8).



susceptible

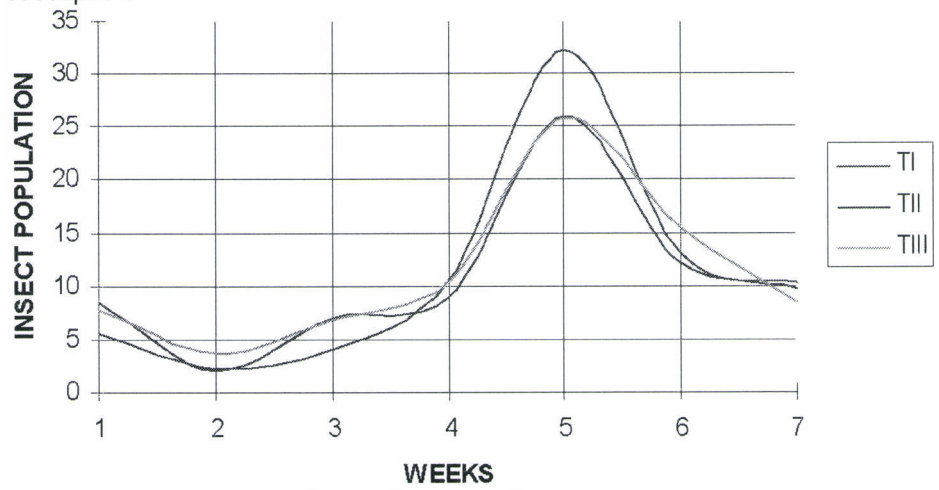


Fig. 7. *Bemisia tabaci* population after transplanting

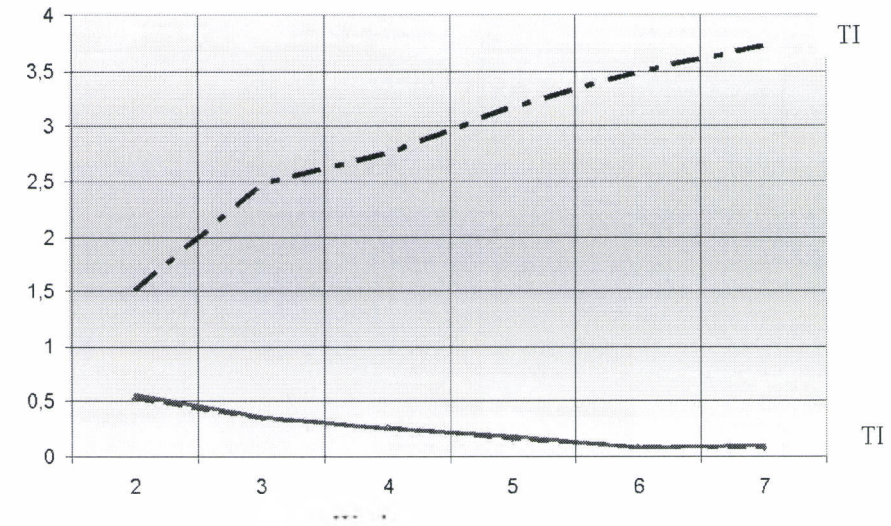


Fig. 8. TYLCV-disease severity after transplanting

The fruit sets were higher (94-95%, respectively) at packages TII and TIII and significant different (P<0.001) to fruit set at classical package (TI) which fell to 45%. Plant development was also lower at TI when comparing to TII and TIII, so production was reduced 80% at TI (Figure 9).

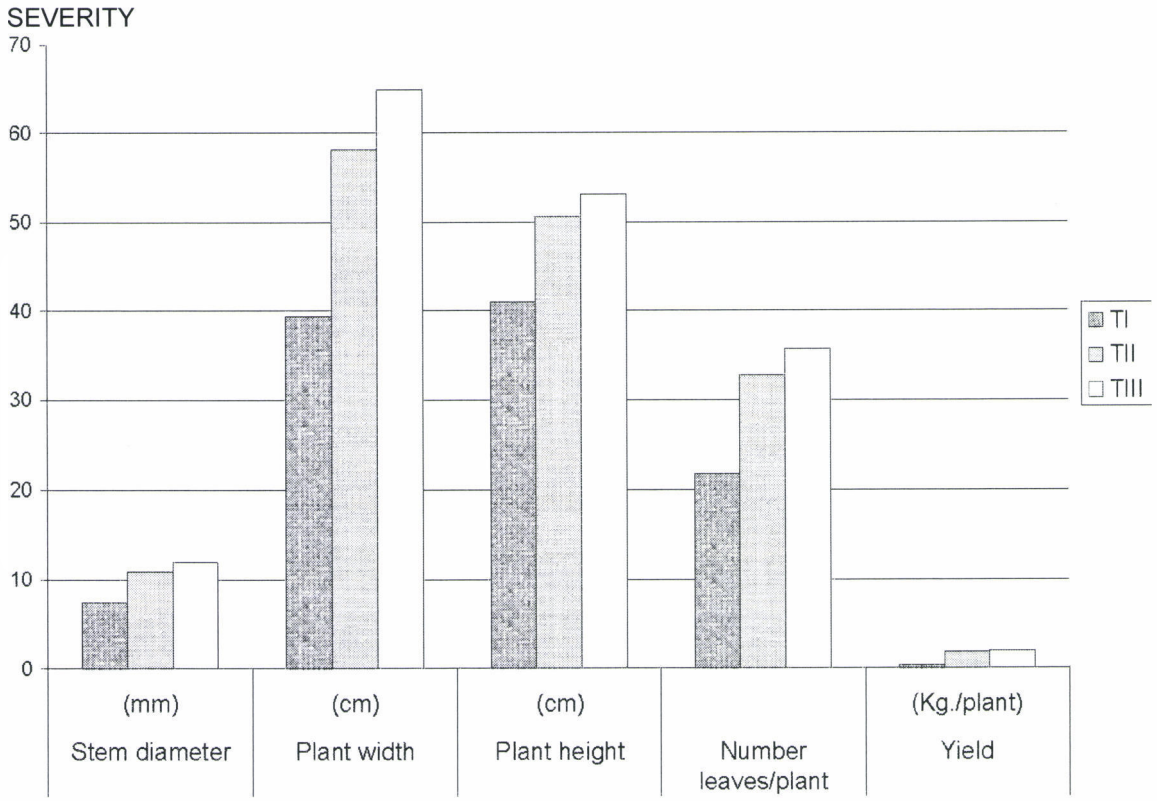


Fig. 9. Plant development and yield at different treatments

The TI treatment (traditional practices + susceptible variety) let a high viral severity which had a negative influence on tomato productivity while treatments TII (traditional practices + resistant variety) and TIII (IPM practices with a resistant variety) contributed to avoid damages with positive economic effects.

CONCLUSIONS

It is possible to enlarge the available tomato cultivars with broad begomovirus resistant ones which also have tropical adaptation. Genetic variability for yield and begomovirus resistance characters were important in the trials carried out and let to an efficient selection of resistant and productive genotypes.

The genotype x environment analysis showed the importance of repetition in variety screening trials, previous to recommend any cultivar in tropical conditions, where weather conditions are often changing, so best results will be obtained when genetic parameters such as yield stabile will be taken into account. The multivariate analyses were useful to classify the genotypes according to their productivity characters.

Resistances to different begomovirus isolates such as: TYLCV-Cuban isolate and TYLCV isolate from the Reunion Island (TYLCV-Mld [RE]), were probed after inoculation by viruliferous whiteflies and grafting, and reported in tomato hybrid parental lines. The Ty-1 gene (carrying resistance to TYLCV from L. chilense) was reported in them by RFLP markers.

A program to produce commercial F1 hybrid seed has been implemented at IIHLD, since seeds of the highlighted hybrids are already available. These hybrids are already being used to grow tomatoes all the year round under sheltered conditions in Cuba.

Using a resistant variety in a correct IPM package bring a solution to grow tomatoes under TYLCV infected areas.

4. Problems encountered
No major problem

5. Technology implementation plan
• ´Vyta´ a Begomovirus resistant variety well distributed in Cuba for tomato open field production. Well adapted to local market. Seeds are available.
• ´LTM 12´ tomato F1 hybrid. It was approved by a national specialized commission and recommended for growing under the "umbrella type" sheltered culture.

6. Publications and papers
TRAININGS
- Jany Fernández. July-August, 2005. Begomovirus integrated pest management on tomato. Natural Resources Institute (NRI), United Kingdom. Partners: Dr. Tim Chancellor and Dr. John Holt.
- Mayte Piñón. October 11, 2005 to February 14, 2006. Tomato breeding for resistance to begomovirus. Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD). Pole de Protection des Plantes, 7 chemin de l'IRAT. Saint Pierre. La Réunion. France. Partner: Dr. Jacques Dintinger.

SCIENTIFIC VISITORS
- Dr. Tim Chancellor and Dr. John Holt from NRI, United Kingdom. March 15-21, 2003.
- Ing. Christian Langlais from CIRAD-FLHOR, Martinique, A.F. May 19-23, 2003.

PUBLICATIONS
Piñón, M. 2002. Nuevos híbridos cubanos de tomate resistentes a begomovirus. Manejo Integrado de Plagas (MIP), CATIE, Costa Rica (61).2002.
M. Piñón y A. Casanova. 2002. Comparación de sistemas para la producción de plántulas de tomate frente al complejo moscas blancas-geminivirus. Manejo Integrado de Plagas (MIP), CATIE, Costa Rica (63):64-70.2002.
Martínez, Y., O. Gómez, M. Quiñones, M. Piñón and D. Fonseca. 2002. Detección de begomovirus en accesiones de Lycopersicon. Plant Pathology No. P-2002-0071-CRA.2001.
M. Piñón, and O. Gómez. 2003. Nuevos híbridos F1 cubanos de tomate asintomáticos al virus del encrespamiento amarillo de las hojas del tomate (TYLCV). Manejo Integrado de Plagas (MIP), CATIE, Costa Rica (68):85-88.
Piñón, M. y O. Gómez. 2003. La lucha genética en el combate de enfermedades del tomate como un elemento de la producción ecológica y sostenible. Agricultura Orgánica 9(1):13-16.
M. Quiñones, D. Fonseca, O. Gómez, I. Miranda, M. Piñón y Y. Martínez. 2003. Optimización y aplicación de la hibridación de ácidos nucleicos no radioactivos para el diagnóstico del TYLCV en el programa de mejoramiento genético del tomate. Protección de Plantas 16(3):156-162.
Sueiro, L., O. Gómez y A. Puertas. 2003. Evaluación de genotipos de tomate en la región este de Cuba. Alimentaria de Tecnología e Higiene de los Alimentos. España (344):47.
Sueiro, L., O. Gómez y A. Puertas. 2003. Validación de la variedad de tomate ´Vyta´ para consumo fresco, con resistencia a geminivirus en áreas productoras de la provincia Granma, Cuba. Alimentaria de Tecnología e Higiene de los Alimentos. España (344):51.
Gómez, O., M. Piñón, Y. Martínez, M. Quiñones, D. Fonseca and H. Laterrot. (2004). Breeding for resistance to begomovirus in tropic adapted tomato genotypes. Plant Breeding 123(1):271-275.
Piñón, M., M.T. Cornide and O. Gómez. (2005). RFLP analysis of Cuban tomato breeding lines with resistance to Tomato yellow leaf curl virus. Acta Horticulturae 695:273-276.
Piñón, M., T. Depestre and O. Gómez. (2005). Cuban tomato F1 hybrids for the sheltered culture system. Iberoamericana-CYTED (submitted).
, M. and J. Dintinger. (2006). Tomato breeding lines resistant to Tomato Yellow Leaf Curl Virus from Reunion Island (TYLCV-Mld [RE]). Plant Breeding (submitted).

SIENTIFIC MEETINGS

• 1st International Symposium on tomato breeding for resistance to begomoviruses. January 20-24, 2003. San Carlos University, Antigua, Guatemala. "A strategy for breeding resistance to begomovirus in tomato".
• XIVth Science and Technique National Forum. January, 2003. Havana, Cuba. "Generalización de la variedad de tomate Vyta".
• VI Reunión de la Red Latinoamericana y del Caribe de Biotecnología (REDBIO). June 3, 2004. Santo Domingo, República Dominicana. "Detection of the Ty-1 gene introgressed from Lycopersicon chilense conferring tolerante to TYLCV in L. esculentum".
• VI Conferencia de las Partes de la Convención de Naciones Unidas para la lucha contra la desertificación y la sequía. Agosto 25-5 septiembre, 2003. La Habana. Cuba. "Creación varietal nacional de tomate"
• 1st International Symposium on Tomato Diseases and 19th Annual Tomato Disease Workshop. June 21–24, 2004. Orlando, USA. "Field performance to TYLCV explanation in new tomato lines".
• Science and Technique Forum. IIHLD. June, 2005. Havana, Cuba. "New tomato processing cultivars resistant to TYLCV"
• Science and Technique Forum. IIHLD. June, 2006. Havana, Cuba. "Implementation of ´LTM 12´a TYLCV-resistant tomato F1 hybrid".

7. Conclusion

Molecular markers work must continue in the progeny (13-8-1 resistant X 13-8-2 susceptible), the possible chromosomic region detection concerning TYLCV resistance in 13-8-1, will allow to build the hypothesis concerning this resistance origin and the implicated genetic factors.

ANNEXES 1 - MEETINGS REPORTS 1

Report of the first meeting BETOCARIB about:

Workpackage A: Identification of begomoviruses and their vectors affecting tomato crops in the Caribbean islands and development of tools for their specific diagnosis.

Identification of wild host species.

During the meeting, we discussed and agreed about the procedures and the protocols necessary to realize the different tasks described in the final document (T01.01 to T01.08, page 7/21).

1) Situation of the different islands for Begomoviruses infecting tomato

The situation of the different island is as following:

- Cuba: Tomato is grown in three different areas. TYLCV occurs everywhere, alone (50%) or in mixed infection with ToHMV (49%). ToHMV is barely found alone (1%).
- Dominican Republic: Tomato is grown in two large areas and fresh tomato is grown in a little isolated area. TYLCV is the only begomovirus detected in the two principal tomato-producing areas.
- Trinidad: Tomato is grown in two different areas on hill sides. PYMV is present in the different tomato producing areas. A strain called PYMV-TT responsible of severe symptoms was cloned and sequenced. It is a recombinant between PYMV-Ve and SGMV-Hn. There is evidence for the occurrence of another strain but their relative importance in tomato crops is not known.
- Guadeloupe and Martinique: Tomato is grown in two different areas. PYMV occurs in all the tomato producing areas. Particular symptoms are observed since October in fields. First results indicate that at least one bipartite begomoviruses is associated to these symptoms.

2) Identification of begomoviruses infecting tomato in the different islands

- The situation is clear for Cuba and Dominican Republic since these countries are yearly surveyed (CENSA in Cuba and Dr Gilbertson for Dominican Republic). However, since only TYLCV is looked for in Dominican Republic, CENSA will use hybridization at low stringency with B component of ToHMV to look for a bipartite in Dominican Republic. If a bipartite is found, then, fragment will be amplified on A and B component with the primers from Rojas and 3-10 clones will be sequenced. The sequences will be compared with the sequences of other begomoviruses in order to identify the virus.
- For Trinidad, Guadeloupe and Martinique, we have to characterize the viruses different from those already known :
- The procedure is the following:
  - Collect 50 samples in different fields
  - Amplify the core coat protein gene (primers from Brown) and fragment on A (rep, IR and 5'CP) and B (5'BL1, 3'IR) component (primers from Rojas),
  - Make restriction enzyme with two enzymes (4 nucleotides site) and compare the result with the profile of the already known virus in the country
  - If there is no difference, then, it can be considered that it is the same virus
  - If there is differences, then clone the different one, select 3-10 clones, sequence them (runs of 500 nucleotides in the two directions) and compare with PYMV and other begomoviruses sequences available in genebank sequences.

3) Identification of *Bemisia tabaci* biotype

3-1) *Situation of the different islands*

What is known is that Cuba and Guadeloupe got B biotype in major proportion, Dominican Republic got B and non-B; Martinique got B and does not have A biotype. Trinidad is believed to have B but it was not much studied.

In this project, we have to collect whiteflies from different ecosystem and to characterize them

3-2) Guidelines for sampling Bemisia tabaci individuals in the different cropping systems of each island

Selection of different ecosystems

The ecosystem will be selected according to the following criteria:

- Geographic isolation to increase the chance for collecting an individual of the non B biotype (in particular, it is suggested to do collections in small islands near the main one)
- Elevation
- Cropping system (monoculture or polyculture)

- Leeward and windward coast
- Dry and wet season
- Any other criteria that may be relevant to your island

Collection of Bemisia tabaci individuals

- In each ecosystem the individuals will be collected on cultivated and wild hosts. The number of individuals that will be tested for each island is about 40. This number can be more or less according to the number of ecosystems identified in each island, eventually depending on the size of each island
- The individuals collected on a single plant or eventually on neighboring plants of the same species should be combined in a single collection tube. Genotyping of the whiteflies will be carried out on one individual of each tube. However, other individuals may be necessary to confirm the result obtained with one individual. Therefore, whenever possible, the number of individuals in each tube should be at least 5.
- It is asked to the collectors to be careful in selecting the whiteflies to reduce the risk of confusion between *B. tabaci* and *Trialeurodes vaporariorum* (greenhouse whitefly).
- Living insects have to be collected and immediately emerged into the collection tubes filled with alcohol (90- 100%), preferably alcohol used in laboratories, "for analysis" or molecular biology quality.
- The tubes have to be completely filled with the alcohol and tightly closed before sending, to avoid that individuals may stick to the walls of the tubes above the alcohol level during the shipping.
- The tubes have to be labelled as described here:
  - Name of sample: first letter of the country and Number (for example, for sample coming from Guadeloupe, it will be G1, G2...)
  - A list of the sample will be joined to the samples giving
    - the place and date of collect,
    - the altitude of the site
    - the host plant,
    - and any other indication which can be necessary

In order to avoid that alcohol leaks during shipping and erases the labelling, it is recommended to write the identifier of each tube on labels sticked onto the tube, and not directly onto the plastic.

The postal address to which the tubes have to be sent is:

Michel Peterschmitt
CIRAD-AMIS, TA 40/02
Laboratoire de Virologie, Programme Protection des Cultures
Avenue Agropolis
34398 Montpellier Cedex 5, France
Tel: 33 (0) 4 67 61 55 87
Fax; 33 (0) 4 67 61 55 05
Email: michel.peterschmitt@cirad.fr

3.3) *Characterization of B. tabaci : genotyping by Random amplified polymorphism DNA (RAPD) in Montpellier*

About 40 individuals of each island will be analysed by RAPD using in each test three internal controls, one individual belonging to the biotype B, one to the biotype A (supplied by J.K. Brown) , and one to a "Jatropha" biotype (J.K. Brown) .

3.4) *Sequencing of a fragment of a mitochondrial gene, cytochrome oxydase I (COI) in Arizona*

All the individuals for which the RAPD profile is different from the B biotype profile, will be further analyzed by sequencing a fragment of the COI gene. Moreover, one individual of each island for which a typical B biotype profile was detected in RAPD, will also be sequenced in the COI gene, as a control.

4) Development of specific tools for diagnosis of the identified viruses

Specific tools are needed to estimate the prevalence of each virus identified in field and in weeds. The diagnosis will be realized by hybridization with PCR labeled probe. This technique enables to treat easily a lot of samples.

- Specific probe. Each partner will have to define the part of the genome that will be use to prepare the specific probe for each virus he has in its country on tomato. The sequence of the intergenic region located at the left of the stem loop on A component (around 150 bp) could be suitable to obtain a specific tool. Cica, Yamila and Uma must verify if this region enables a specific diagnosis. Douglas Maxwell will send to Cica, the thesis of Jamie Potter for the direct labeling of PCR fragment. Cica will send a synthesis and the protocol to Michel, Yamila, Umaharan, Judy. There is a kit from Amersham, which enables to do that. Martinez must send the reference.

5) Estimation of prevalence of each virus in tomato crops at the ecosystem level

5-1) *Collection of tomato samples*

- If only one virus occurs in the country, incidence will be estimated by counting symptomatic plants

When? One survey will be done at the beginning of the cropping season and one at the end of the cropping season of each country
Where: in the different ecosystems defined in the main tomato producing areas. The ecosystem can be chosen as defined in 3.2
How many fields? This must be defined by John Holt according to the precision he needs for the epidemiological study. Statisticians should define it

- If more than one virus occurs in the country (at least Cuba), we have to estimate the relative incidence of the viruses in the different ecosystems

When? One at the beginning of the cropping season, one at the end of the cropping season of each country
Where : in the different ecosystems defined in the main tomato producing areas
How many fields and how many samples per field? This must be defined by John Holt and statisticians according to the precision which is needed for the epidemiological study

5-2) *Detection of begomoviruses by dot blot hybridization*
Dot blotting of tomato samples: Yamila Martinez (CENSA) proposes a very quick protocol to extract DNA from tomato sample before blotting Yamila has to send the reference of this protocol. Diagnostic will be conducted by Hybridization as defined in 4

6) Identification of weeds carrying the targeted begomoviruses

6-1*) Weeds species that should be collected*

Cuba and Dominican Republic have a list of weeds known to be infected with TYLCV and /or ToHMV. Trinidad and Guadeloupe also have a list of weeds carrying PYMV. In order to define the species of weeds that should be collected , each participant will send to Cica its list of the species which are host of the begomoviruses infecting tomato in his country. Cica will combine these lists with the other species known to be host of begomoviruses in tropical area and send to each country the complete list.

Remember that the weeds, which will be found to be infected with tomato begomoviruses, should be collected and transplant in pots for the transmission tests with B. tabaci biotype B. So, It is better to identify them in field or at least to identify the field very well.

6-2) *Protocol of DNA extraction from weeds*

Concerning the detection in weeds, the problem is that some plants give sticky or phenolic extracts which do not enable the diagnosis of begomoviruses. We have to define the best protocole for begomoviruses detection in each species.
Different protocols have been already tested by the different teams (Dellaporta modified or not, Doyle and Doyle, Kobeyashi, Qiagen DNeasy plant minikit). Umaharan (UWI) reported that Kobeyashi was the best one. Uma has to send Kobeyashi's protocol to Douglas, Cica, Yamila and Michel. Uma will test these protocols on different species of weeds for the DNA quality (RAPD, restriction digestion, degradation of DNA) and will propose the best ones. The participants CENSA (Yamila) and CIRAD (Cica) will test them on infected weeds to define the best protocol for each species.

6-3) Blotting and hybridization

Each sample will be blotted on two membranes (if only one begomovirus occurs on tomato in the country) to three membranes(if 2 different begomoviruses occurs on tomato).
The first membrane will be hybridized with a generic probe allowing the detection of a begomovirus (old world and new world begomoviruses)
The others will be hybridized with specific probe for tomato infecting begomoviruses.

7)Definition of a generic probe to potentially detect all the new world begomoviruses and differentiate old world from new world begomoviruses.

Rojas et al developed generic primers in 1993 for the general detection of begomoviruses. Since this time, many begomoviruses have been fully sequenced and many sequences are available on Genebank. This project represents an opportunity to try to define new tools more appropriate for potentially detecting all the NW begomoviruses and differentiating old world and new world begomoviruses (OWB and NWB) and propose it for general diagnosis. This tool would be a contribution of the project to the diagnosis of begomoviruses.
NWB mostly have A and B component, but rarely an A component alone.The new world A components (Anw) are approximately 2600 to 2680 nucleotides. OWB have A component or A and B components. The

old world A components (Aow), are around 2800 nucleotides. The differences of length between Aow and Anw are due to a size difference of the nucleotide region between the conserved nonanucleotide and the start codon of the CP gene.

We have to find sequences of primers that would allow to potetnially detect all the NWB and primers that would only detect the OWB The primers of Rojas were designed with the sequences available in 1992. Since this date a lot of new sequences were published.
Primers:
We propose that Yamila, Douglas, Michel send to Cica the name and accession numbers of different begomoviruses. Cica would choose around 10 begomoviruses each representative of a cluster.
Phylogenetic trees can be found in

- http://www.danforthcenter.org/iltab/geminiviridae/sequences/america_files/america.html

- Faria, J. C., Gilbertson, R. L., Hanson, S. F., Morales, F. J., Ahlquist, P., Loniello, A. O., and Maxwell, D. P. 1994. Bean golden mosaic geminivirus type II isolates from the Dominican Republic and Guatemala: Nucleotide sequence, infectious pseudorecombinants, and phylogenetic relationships. Phytopathology 84:321-329.

❑ Douglas send a proposition the 22 of March 2002 for Designing general (degenerative) primers for New World begomoviruses

There is no general agreement as to the major clusters or clades among those of us who work on taxonomy, but the following will cover the diversity of begomoviruses in the New World.

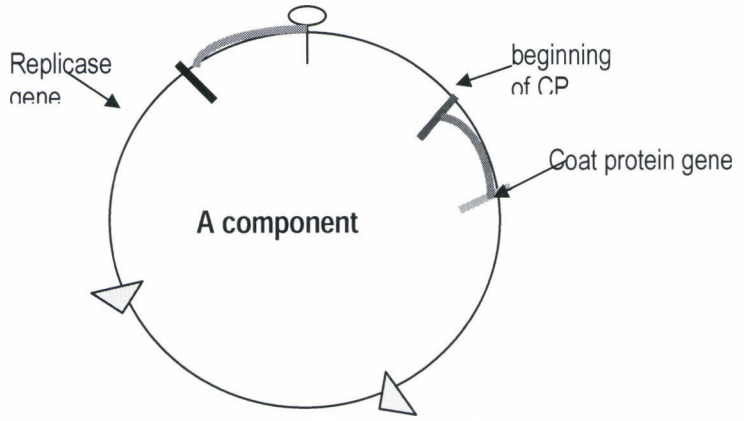
- Clade: Pepper huasteco yellow vein virus X740418
  This virus is a recombinant between new and old world. The Rep gene is most similar to old world begomoviruses. This has become an important tomato-infecting begomovirus in Mexico.
- Clade: Squash leaf curl virus (extended host range) M38183
  There are many viruses in this clade and they have can be recognized by a deletion in the Rep gene, which only occurs in viruses in this clade. The only virus in this clade that infects tomato is Tomato serve leaf curl virus from Central America. Other hosts of viruses in this clade include: melons, papaya, beans, cucumbers, and some weeds.
- Clade: Bean golden yellow mosaic virus -[GT] M91604
  This is a very distinct clade with only BGYMV isolates from Central America, southern Mexico, and Caribbean.
- Clade: Potato yellow mosaic virus (use sequence from Urbino) and also PYMV-TT (AF039031)
  As you know these isolates are found on tomato.
  Similar to several viruses in weeds in Jamaica.
- Clade: (sometimes called the Abutilon clade and includes ToMoV and many other viruses)
  Tomato Mosaic Havana Virus Y14874
  Viruses similar to ToMHV have been found in Jamaica and Honduras.
- Clade: not really a distinct clade and might be included in the Abutilon clade, but should be considered: Sida Golden Mosaic Costa Rica Virus X99550
- Clade: not really a distinct clade but might be considered as one: Tomato leaf crumple virus (chino del tomate) AF101476

Strategy:

- Michel would then align the replicase gene of these viruses and look for a conserved region to define primers. When you do the sequence alignments, you might include two viruses from the same clade listed above, eg for the squash leaf curl virus clade use SqLCV and may be pepper golden mosaic virus, since it goes to tomatoes.
- Yamila would align the coat protein gene and look for a conserved region to define primers.
- For the detection of NWB, the primer would be chosen in the core coat protein, or one in the CP and the other in the Rep depending on the purpose of the amplification.

❑ For the specific detection of OWB, a primer should be designed in the region that only exists in the OWB between the nonanucleotide and the start codon of the CP gene. .

Probes: Douglas will provide the core coat protein of TYLCV from Dominican Republic and Yamila will provide the IR of TYLCV from Cuba as probe for old world begomoviruses. Uma will provide the left part of the intergenic region (IR) of PYMV as probe for new world begomoviruses.
Each team will verify if these probes can differentiate OWB from NWB (PYMV, TYLCV, ToHMV, at least).



8) Test of transmission between different biotypes and begomoviruses
In Cuba and Guadeloupe, the transmission efficiency will be carried out on one reared population of B biotype individuals. This transmission rate will be compared to the transmission rate of the non-B biotype populations, if any. For the detail of experimentation, see the procedure "Proc BETOCARIB A Tr Tom-Tom 2002-01.doc"

For islands in which several begomovirus species or strains are infecting tomato, the transmission rate has to be determined with all of them.

9) Transmission biotest by B. tabaci from weeds to tomato and from tomato to weeds
This part has not be developed during the meeting. We propose that Cica, Yamila , Maria, and Uma who will have to make the transmission biotests exchange email to define a common protocol. We propose some ideas to begin the discussion

- Transmission biotest by B. tabaci from weeds to tomato
  - We must first collect the weeds infected with tomato infecting begomoviruses and maintained them in pots (see 6.1). Some species are not easy to transplant so many plant should be collected before the beginning of the tests. If necessary, the presence of tomato infecting begomovirus should be verified just before the transmission tests.
  - Maybe the whiteflies will not feed on some weeds and after 48 hours of acquisition, they will be dead. So, during the acquisition period, we should observe the behaviour of the whiteflies and if necessary test different acquisition periods.
  - The inoculation period on tomato should be long enough in order to have the best opportunities to obtain a transmission. So we can leave the whiteflies 4 days on tomato plants.
  - We have to make these transmission tests with the most important tomato variety used in the country.
- Transmission biotest by B. tabaci from tomato to weeds
  - We must first collect some seeds of weeds which were identified as possible host of tomato infecting begomoviruses. Should we collect the seeds ourselves in field in each country and sow them or should we ask some seeds of each species? What do you think Uma? It seems that you have collection of seeds of weeds? Is it true?
  - Same remark for the inoculation period as before
- How may repetition should we do, with how many insects per plant? These entire questions will depend on the capacity of rearing insects.
- Are we interested in the rate of transmission or do we just want to have an answer YES/NO. If it is no after the first tests, should we do it again?

- We can also imagine to work with mass inoculation: we put the infected plant in a cage with the whiteflies and after an acquisition period, we add the plant test and shake the plants to assure a repartition of whiteflies; we leave the plants during a week.
- Plants should be tested 1 month after inoculation for tomato and more than one month in weeds.

Promises : Who should do what??

1. Each participant will send to Cica its list of the species, which are host of the begomoviruses infecting tomato in his country.
2. Cica will combine these lists with the other species known to be host of begomoviruses in tropical area and will send to each country the complete list
3. Uma has to send Kobeyashi's protocol to Douglas, Cica, Yamila and Michel.
4. Yamila has to send the reference of the rapid protocol of extraction for blotting tomato samples (derived from Dellaporta)
5. John should tell us how many fields and how many samples should be collected per field for the study of the prevalence of begomoviruses in the different ecosystems This must be defined with statisticians according to the precision which is needed for the epidemiological study
6. J.K. Brown will supply individuals of Bemisia tabaci biotype A and "Jatropha" biotype for the RAPD)
7. Douglas Maxwell will send to Cica, the thesis of Jamie Potter for the direct labeling of PCR fragment. Cica will send a synthesis and the protocol to Michel, Yamila, Umaharan, Judy.

ANNEXES 1 - MEETINGS REPORTS 2

REPORT OF THE FIRST MEETING AND THE TRAINING SESSION OF BETOCARIB AT TRINIDAD
13-17 October 2003

Work package A - Tasks

T01.02	Identification of begomovirus infecting tomato
T01.03	Identification of *Bemisia tabaci* biotype
T01.04	Development of tools for diagnosis of the identified virus
T01.05	Estimation of incidence in tomato crops
T01.06	Identifying weeds carrying the targeted begomovirus
T01.07	Transmission biotest by *B. tabaci*
T01.08	Tests of transmission between different biotypes
T01.09	Training session

During this first meeting, the different teams have presented the results obtained from the beginning of the project and corresponding to the tasks T01.02 to T01.09. Some technical questions were identified and treated during the Training Session. Then a general discussion was conducted in order to identify and precise the further tasks necessary to finish the work package A in the best conditions.

1) Presentation of results

The principal results are summarised in Table 1. The annual report and the 18-months report were used to inform the participants of the results from Cuba. The principal tasks were achieved or are in progress for all the teams.

A visit in the different tomato producing areas at Trinidad was organised and was very interesting regarding the own particular problematic of this island. PYMV-TT is now called PYMTV: potato yellow mosaic Trinidad virus, as recommended by International Committee of Taxonomy of Viruses (Fauquet et al, 2003, Archive of virology, 148: 405-421). Symptoms caused by PYMTV are yellow mosaic, leaf size reduction. They look more severe than those due to PYMV in Guadeloupe and Martinique.

The questions resulting from the meeting and submitted to discussion were the following:

1. The virus identified in Guadeloupe and Martinique presents a gap of 30 base pair in the intergenic region on the left side of the origin of replication. This is not common among other TYLCV isolate and it must be verified that this is not an artefact of PCR amplification. Direct isolation of viral genome should be done and cloned to verify this result, combining with PCR amplification using other TYLCV isolates (from La Reunion or other...).
2. The PYMTV strain is a recombinant of PYMV and *Sida golden mosaic virus* SiGMV. PYMV was no more found in infected tomato collected 5-7 weeks after plantation in field. It is proposed that some infected plants are collected early in fields at the beginning of the infection (2-3 weeks after planting) in order to be sure that PYMTV is the only one infecting tomato plants in Trinidad. In fact, because PYMTV is the dominant viral infection it may not be possible to detect PYMV in mixed infection with PYMTV even if it is present.
3. In the Dominican Republic, incidence of TYLCV was mainly established in the processing tomato areas but not in the centre of the country, which is a

salad tomato area. The processing tomato areas are submitted to the free crop period and high chemical treatments in order to control the TYLCV. Growers in the salad tomato production area are not submitted to these regulations due to the small tomato producing fields. They use no resistant varieties and less chemical treatments compare to the intensive processing tomato producing areas. This could lead to a very different situation of virus and vectors population from what was described in processing tomato areas by Salati *et al* in a survey between 1998 and 2001(Phytopathology 2002; vol 92, N°5 pp. 487-496). It was recommended that a survey should be realised in this area in order to collect tomato plants and Bemisia samples. The same protocol as the one used in Guadeloupe during the survey (annexe 5 annual report 1) could be used to collect additional information about the technical management of this production useful to perform/evaluate/improve the ecosystem model.

4. Cuban team has detected TYLCV in *Cucurbita pepo*. This is the first report of TYLCV in a *Cucurbitaceae*. Because of the economical importance of cucurbits, this should be confirmed with transmission tests from tomato to *C. pepo* and from *C. pepo* to tomato.
5. TYLCV was detected in alternative hosts in Dominican Republic only when a lot of DNA extract was used in the diagnostic test (Salati et al., 2002 Phytopathology; vol 92, N°5 pp. 487-496). So, we can consider that this task should not be done again for Dominican Republic. However, we wonder if the results obtained in such conditions (with large amount of DNA extracts) allow considering that these species are efficient hosts of TYLCV in natural conditions. Large DNA amounts of plant DNA extract will be used in PCR mixture in Cuba, and Guadeloupe to try to obtain virus amplification from weeds already listed to be host of TYLCV. If positive viral amplification is obtained, transmission tests will be tried to verify the efficiency of these hosts as viral reservoir.
6. Primers, which were designed for the specific diagnostic of TYLCV, were able to detect PYMV in Guadeloupe using protocol as defined in literature. For example, we obtained PCR amplification from TYLCV and PYMV with primers IR255/IR2353 using the usual annealing temperature of 60°C, when specific TYLCV PCR amplification requires a 68°C annealing temperature. It should be verified that the conditions of use of these primers are specific enough. Exchanges of technical information should be done between CIRAD Guadeloupe and CENSA Cuba who use these tools.
7. Difficulties were encountered in Guadeloupe since July 2003 to hybridise with the several probes already used before (AlkPhos from Amersham). It seems to be related to the labelling of the probes and the new batch of kit used. Since the manufacturer did not have any problem with this product, some tests should be done to identify the problem in its application. At this moment all the diagnostic planned in Guadeloupe is waiting for efficient probes. If the problem persists, the dig labelling system (from Roche diagnostic) could be use in replacement.

2) Results from the training session

This training sessions was focused on different points which were identified as key points between the participants in order to really interact to solve them. They concerned the following aspects:

- Improving DNA extraction with Kobayashi's Protocol in order to improve detection of begomoviruses in weeds
- Improving the diagnostic of begomoviruses by testing new primers for detection of begomoviruses from old and new world.

2.1) Partner 4 had worked on the extracting DNA protocols in order to propose the most suitable to detect Begomoviruses in weeds.

Kobayashi's protocol (annexe 1) was applied on several weeds (*Sida, Chorchorus, Meremia, Euphorbia*) and on PYMTV infected tomato plants, all collected during the field trip. The basic protocol was modified as following: 0.4g of leaf was used for the extraction; 1 to 3 washing with buffer 1 was done after the first centrifugation for all the samples which gave a very sticky supernatant. Very short centrifugation was done after DNA precipitation with cold isopropanol to prevent the co-precipitation of phenolic components. The phenol extraction was not done at the end of the protocol.

PCR was then conducted on these DNA extracting samples using primers MP16-MP82. PYMTV cloned virus was added to healthy plants DNA extracts of *Rhynchosia* and *Sida* to verify whether PCR inhibitors resulted from this Kobayashi's extracting protocol. PYMV from Guadeloupe and TYLCV Reunion cloned viruses were the positive PCR controls.

Results: Amplification was only obtained for the positive controls and PYMTV. Adding cloned virus in healthy weeds DNA extract did not allow the expected amplification, meaning that PCR inhibitors are still present in the DNA extraction.

Discussion:

1. This protocol must be very efficient for plant DNA extraction but it is not sure that it is well appropriate to virus DNA extraction. Begomovirus size is around 2.8 kb and this size of DNA may not precipitate with short centrifugation.
2. It may be better to work on less than 0.1g of total leaves in order to modify the ratio "begomovirus DNA/ total DNA plants" for plants which contain very sticky components.
3. Several ways should be explored in order to optimize the begomoviral DNA extraction from DNA plants such as
   - Using Ikegami's protocol to isolate viral DNA directly from plant. Since DNA has a similar size compared with plasmid DNA, protocols, which allow plasmid isolation from bacterial DNA, could be tried.
   - Molecular hybridization could be used to control the lost of viral DNA during the different steps of the DNA extraction protocols
   - Protocols to extract viroïds, which have small genome, using lithium chloride could be analysed.
4. IC-PCR was found to be performing to detect begomoviruses in weeds. This should be tried by Guadeloupe (Partner 1) and Cuba (Partner 6).

2.2) New set of primers for general diagnostic of begomoviruses

The two primers designed by Michel Peterschmitt (Partner 1) were tested during this lab session. Each newly designed primer (located in the rep) was combined with PCR1c496 designed by Rojas et al, (1993) as complementary primer. Old world (TLCV India, TYLCV Guadeloupe) begomoviruses and New world (PYMV, PHV) begomoviruses were tested with these set of primers in a PCR gradient apparatus. Temperature ranging from 48 to 62°C was tried.

Results: one amplification only was obtained with PYMTV at 48°C.

Discussion: maybe the primers were not compatible (annealing with PCR1c496) or they were too degenerated. Yamila Martinez (Partner 6) has proposed other primers located in the pre-coat gene for diagnostic of old world viruses. These primers should be tested.

3) Conclusion of the first meeting for WPA and the Training Session

1. Begomoviruses identified in the five islands are PYMV and TYLCV (Guadeloupe and Martinique), PYMTV (Trinidad) and TYLCV and TOHMV (Cuba) and TYLCV (Dominican Republic). Any or few virus diversity was observed. The B biotype of *Bemisia tabaci* seems to be preponderant in all these islands.
2. The incidence of begomovirus in each island was estimated and this revealed that begomoviruses are the most important viral constraint of tomato in the Caribbean. The introduction of TYLCV in this area has lead to increase damage in this culture.
3. No alternative host was found for PYMV or PYMTV, suggesting that tomato is the principal host of these viruses. Many hosts were found for TYLCV in Dominican Republic and 2 hosts were found for the same virus in Cuba. However, considering the conditions in which these results were obtained, transmission test should be tried to verify whether the virus could be transmitted from these plants to tomato.
4. Immunocapture PCR tested by Umaharan et al (2003) seems to represent a good alternative to obtain PCR products for cloning and sequencing begomoviruses from weeds. However, molecular hybridisation is an adapted method for routinely begomovirus diagnostic, at the genus level or for specific diagnostic.
5. New set of primers were tested in a first attempt and did not give expected results. Other primers, defined by CENSA (Y. Martinez), will be tested soon by the several teams.
6. A survey should be done in the salad tomato producing area of Dominican Republic to confirm the absence of PYMV, which was detected in 1995 by Polston et al, 1998 (Plant Disease, 82, 126), establish the incidence of TYLCV in a not intensive tomato producing system and to identify *Bemisia* biotypes in this area.
7. The first results of the projects have already been published by the different teams (cf 18 months report). New subjects of publication identified to promote the best interactions between the different teams of the project.
   - Partner 1 and 4: relationships between PYMV in Guadeloupe/Martinique and PYMTV in Trinidad in terms of strains or recombinant each other or with other begomoviruses.

- Partner 1, 4 and 6: Primers to specific detection of NW and OW begomoviruses.
- Partner 1, 4 and 6: weeds host of TYLCV and PYMV and methodology to detect begomoviruses in weeds.

8. In order to prevent the progression of TYLCV in the Caribbean islands from Guadeloupe to Trinidad, it will be important to conduct survey in the small islands in between. A proposal was written on by Partner 4 and 1 during the meeting and submitted for funding to CARDI.

	Guadeloupe	Martinique	Trinidad	Cuba	Dominican Republic
Virus characterisation	PYMV occurs since 1993 TYLCV newly identified since 2002. The strain is very closed to that which occurs in Cuba and Dominican Rep.	PYMV occurs since 1992 TYLCV newly identified since 2002 Same strain as the one described in Guadeloupe	Only PYMTV was identified	TYLCV is the dominant virus. ToMHV seems to regress	TYLCV is dominant in the processing tomato area. No other begomovirus was found described?
Begomovirus diversity	No diversity was found for the two viruses with PCR-RFLP	No diversity found in 40 isolates of TYLCV and PYMV with PCR-RFLP	No diversity was found . PYMTV has replaced the referenced strain of PYMV which could be detected in 1995	No diversity was found for TYLCV with study of the secondary structure	The principal begomovirus was identified as TYLCV (Salati et al, 2001)
Characterization of Bemisia	35/37 samples of Bemisia were identified as B biotype 2 samples with non B typical patterns will be sequenced for COI	Samples are being collected	Samples are being collected	Sequencing of COI being done	7 samples collected in processing tomato areas were identified as B biotype with RAPD
Incidence of begomoviruses	TYLCV and PYMV are principally detected in mixed infection in all tomato producing areas. 50-100% in Grande Terre, Basse Terre and Marie Galante	North Caribbean coast TYLCV and PYMV 50-100% North Atlantic coast PYMV 10-20% Some source of TYLCV appeared in 2003. No begomovirus in the south of the island	PYMTV occurs in all producing areas West: 70-100% East 43-80% Caroni 20-100%	100% of 1300 samples collected in 2003 were TYLCV infected.	The incidence is being estimated by CENSA Cuba
Begomoviruses in Weeds	No host was found for PYMV No host was found for TYLCV even in the list of host established from the previous results	Not done	No host found for PYMTV, even those previously cited in Umaharan et al.1998	TYLCV was detected in *Euphorbia* and one Squash plant. This is the first report of TYLCV in a *Cucurbitaceae* species.	A study was done in 2001 by Salati et al, 2001, identifying 14 different species as hosts of TYLCV

Workpackage B – Tasks

T02.02	Target survey to validate the agrosystem model
T02.03	Epidemic development and dynamics of B. tabaci in 3 representative sites of the Caribbean islands
T03.04	Identification of biotic and abiotic (climate) factors prevalent on epidemics
T03.05	Development of crop model of epidemics.

Ecosystem model

The methodology for the epidemiological surveys carried out in Basse Terre and Grande Terre (C Pavis), and the results of the statistical analysis of the surveys (J.Holt) were presented. General linear models were used to identify which variables were associated with disease incidence, either positively of negatively. By removal and replacement of each variable and noting the change in deviance, a statistical model containing all the significant, and only the significant, variables was obtained. Models were fitted separately for Basse Terre and Grande Terre and the two ecosystem models so obtained show which properties of the ecosystem were associated with begomovirus disease. The variables which had a negative association with disease incidence and/or severity in both parts of Guadeloupe were identified as being of particular interest as they may suggest variables that could be manipulated to reduce disease incidence.

In addition to the epidemiological surveys carried out under Workpackage B, a survey was carried out in Trinidad (P Umaharan) which included some epidemiological information. The survey involved 125 tomato farms in 8 areas (+1 in Tobago) as well as dicot. weeds in tomato areas and in non-tomato areas. In Trinidad there are about 450 ha under tomato, involving 350-400 farmers.

In Trinidad, cultivation is mainly in open fields, some greenhouse, some 'grow-box'. Production is year-round but with a dip in August/September. Typically, year round production occurred in clustered open fields. Average yields are 6t/ha. 45% of farmers used imidacloprid and other sprays. PYMV occurred in 48-78% of cases. Now only the recombinant form is found which has replaced the original. TEV and TMV were present but to a lesser extent than PYMV. There was high variability in begomovirus incidence within a county. Nurseries were virus-free. The Bemisia B-biotype was consistently the biotype found in the tomato areas. No PYMV was found in any weeds. Some statistical analysis was carried out and tomato-growing location was the only significant variable affecting disease incidence. It appeared that locations with more scattered cultivation had lower infection

Field work on disease epidemiology and Bemisia population dynamics

The following experiments have been carried out, are in progress, or are planned. They were designed in part, to test the effects of some of the important variables identified using the ecosystem models and in part, to provide information about disease epidemiology and the population dynamics of Bemisia. 1 to 4 below were described during the workshop (C. Pavis).

1. Year round Bemisia population and infectivity monitoring. This includes monitoring of primary inoculum based on the disease incidence that results when 50 plants in pots were placed in the field for one week. The plants are replaced weekly, so a year-round measure of inoculum pressure is obtained. Bemisia are monitored by mast traps, and by yellow traps in a plot of pumpkin. For the corresponding period, weather records are also taken: temperature, humidity, wind direction & speed, rainfall. In addition records of chemical treatments, crop periods and crop areas are recorded. At Godet, these data cover the two-year period 2001 to 2003; at Vieux Habitant, the one-year period, 2002-2003. In the latter, Bemisia monitoring is by mast traps only. The work is carried out on the CIRAD station which is some distance from the main tomato-growing area in Basse Terre.

2. To establish the gradient of dispersal of whiteflies and the associated effect on the spatial spread of disease, a 'Disease gradient experiment' experiment was carried out at Godet. The source plot was a large plot of PYMV- infected tomato used for variety screening trials. Two blocks of 50 plants in pots were stationed at 100 and 250 m downwind from the source plot in a pathway cut out of a large area of sugar cane . Disease incidence and whitefly number were recorded on several occasions in the two blocks. More whitefly and higher disease incidence were found in the 250m block than the 100m block. This perhaps suggests that whitefly dispersal may not follow a simple gradient or that local effects influenced the arrival of primary inoculum from other sources
3. Transmission tests using tomato have shown that the minimum begomovirus (PYMV) acquisition period is 30 minutes and the minimum inoculation period is 3 hours. The latent period in the whitefly vector is 15 hours. The infectivity of the vector persists for at least 8 days. Disease symptoms appear in susceptible variety 8 – 10 days after inoculation. This may also represent the latent period of the virus in the host plant. In a resistant variety, symptom expression can be delayed by 8 to 10 days (Cica Urbino). Whitefly life span is 15 to 30 days on melon under laboratory conditions. Whitefly fecundity has been measured (and r_m calculated) on different melon varieties under different climatic conditions. Whitefly generation time is about 3 weeks. This work was not part of BETOCARIB but provides useful parameter estimates for the crop model.
4. A 'Barrier experiment' was carried out to test the effect of a 1.2 m high whitefly-proof fence surrounding around the plot. In a first experiment (in the dry season, an insecticide-impregnated yellow band was also incorporated on the inside of the fence. Though most Bemisia movement is known to be close to the ground, the band is designed to attract and kill any whiteflies that fly over the fence into the plot. In a second experiment (in the wet season), the fence was used without the yellow band. Disease progress was recorded weekly in replicated plots, control plots having no fences. The results for the first experiment were impressive with disease progress curves being delayed by at least two weeks in the fenced plots. In the second experiment, either due to the lack of yellow insecticide bands or the differing inoculum pressure in the wet season, no effect of the fences was demonstrated. Clearly, this technique has the potential to curb begomovirus disease progress at least under some circumstances. An important question remains as to whether the delay in disease progress obtained translates into improved tomato yield. Disease progress curves were obtained separately for TYLVC and PYMV in the control plots. These observations may be informative about any antagonistic or synergistic interactions between them.
5. Planned for the coming season, an experiment to test the effects of Imidacloprid as a seeding dressing, a post-planting spray and a combination of the two, will be carried out in Cuba. The results from the epidemiological survey and previous trials in Cuba have given rather ambiguous results concerning the effect of insecticide treatments to control whitefly. It is hoped that the experiment will help to clarify the impact of insecticide-based vector control in the TYLCV disease progress.

Crop model

Initial ideas for structure of a the crop model were presented. Some possible questions for the crop model were identified during discussions and during field visits. Some questions concern disease progress in a single crop to establish the relationship between whitefly number, infectivity and disease progress and also allow the effect of management interventions, such as chemical control or whitefly-proof barrier fences, designed to reduce whitefly number, to be evaluated. Aspects of these questions could be examined in some detail. For instance, the effect of suppressing whitefly number at different times and for different durations could be examined.

Other possible questions were raised which would require a model spanning more than one crop, and in some cases representing space (i.e. multiple fields) in some way, e.g. What would be the effect of host free periods of different durations on long-term infection pressure

and so disease incidence in a system? What might be the effect on disease in a system of tolerant varieties that nonetheless act as virus sources? Compare this to resistant varieties?

Some findings from workpackage A had salient results for modelling. Most important was that, in Guadeloupe at least, none of the weeds tested carried the same begomoviruses as those in Tomato. From Cuba, there were second hand reports of TYLCV in at least some weed samples but no details were available. If weeds can be regarded as an unimportant host for tomato begomoviruses, then this obviously impacts on the form of model that might be built.

A visit to a tomato growing area in Bonne Aventure raised some interesting questions . A succession of three tomato crops had been planted in March/April, July and Sept/Oct. This area only started to suffer virus problems only the previous year(?). The area is relatively isolated and infection may have been imported on seedlings. According to the farmer, in the current year the first crop had no virus problems, in the second infection started (was first visible) at eight weeks (and the crop was currently 14 weeks old). The third crop, about 10 days old at the time of the visit already showed about 20% infected plants. The farmer used an imidacloprid seed dressing, a drench 1 week later and planned a second spray or drench at 10 –15 days. Despite these measures the final crop of the season was clearly infected so early as to suffer extreme losses. The pattern of cultivation was discussed and some old tomato crops were located up wind of the new crops. The farmer had no control over these, as they belonged to someone else.

A possible question for a crop model relates to the pattern of crops in time and space and the consequences for disease in the system. The results of this could relate, in a general way, to the potential impact of schemes of collaboration between growers in an attempt to reduce disease. To do this would probably require rather a lot of assumptions about the processes of whitefly movement and how they are affected by wind direction and natural barriers such as non-hosts.

At a field location close to Curepe (?) were a large amount of vegetable production takes place, a severely infected field with 100% incidence was located. Disturbing the plants also suggested an average of at least 1 to 2 whiteflies per plant. Next to this plot was a plot of cabbage with very large numbers of whiteflies – around 5 on a high proportion of leaves, possibly as many as 50 per plant. The next plot was a plot of younger tomatoes, all of which were also infected. This highlighted the potential effect of whitefly hosts which may carry vastly more vectors than tomato.

Particularly in cropping systems which have a mixture of tomato (the main, and possibly only significant, virus and whitefly host), whitefly hosts (cabbage, aubergine, cucurbits, etc) possibly mixed with non-hosts, important issues exist as to the effect of differences in this crop mixture on disease levels. A model, which allows changes in the cropping system mix to be examined, may be of value.

Whatever the form of the modelling exercise, the important thing to assess is the delay in infection achieved under different circumstances and through different interventions. This point was especially highlighted during the field visits, were several cases of 100% infection still had high yields because infection was sufficiently late.

Data required to parametrise and test the crop model. The model parameters will of course depend on the form of the model but knowledge of the following is likely to be required. For PYMV, experiments already carried out at INRA, Guadeloupe, can provide information about minimum acquisition and transmission times (30 mins and 3hrs on tomato) as well as the latent period in the vector (15hrs). Persistence of PYMV in the vector is at least 8 days.

Whitefly can live for 15 – 30 days under laboratory conditions. There are also data on whitefly fecundity and r_m under different climatic conditions on different varieties of melon.

A current unknown is the latent period in the host, which like several of the other parameters discussed above, is likely to vary between resistant and susceptible varieties. On susceptible varieties, PYMV (?) symptoms appear 8 – 10 days after inoculation. On resistant varieties this is delayed by a further 8 – 10 days.

J. Holt and C. Pavis discussed the possible effects of host area on the contact rate between whiteflies and tomato plants. As the plant grows the contact rate may increase. Information about details of tomato growth may be available from the 'STICS" model being adapted for tomato by Nadine Brisson of INRA.

Some data have already been collected to allow comparisons between the predictions of a crop model and disease progress in the field. Notably, the experiment to test the effect of whitefly-proof fences ('barriers'). Further data are needed to test the crop model, e.g. a series of measurements, perhaps weekly or every 2 weeks, during the early part of the crop period up to disease saturation. Counts of incidence, whitefly number (an estimate of the number on plants is best but a relative measure using traps is useful too). Records of any management interventions such as insecticides, variety and details of crop environment (to help infer something about relative infectivity of immigrating whiteflies). It is useful to test a model over as wide a range of conditions as possible and so a series of data sets with, for example, susceptible and resistant varieties in low and high inoculum pressure areas would be very helpful. A further useful way to extend the range of conditions is to include an experimental treatment in the design. In the first trail of the barrier experiment, for example, the two treatments had very different disease progress curves.

Discussion of further possible experiments for Workpackages B & C
Some discussion took place on further possible experiments that might be done with the dual role of providing further useful data to compare with the crop model as well as to test experimentally control measures that may have some potential. Thus, the experiments would have relevance to both workpackages B & C.

Weeding experiment – effect of weeding, or perhaps critically, the timing of weeding – in the workshop it was suggested that initially, a lab approach may be useful to test weed to tomato transmission under controlled conditions.

There was some discussion of UV reflective mulches. It was suggested that the literature on this was quite large and that it would be worth reviewing this before embarking on an experiment.

Action points
1. Decisions are needed about experiments with the dual role to provide crop model 'validation' data for Wp B and test IPM components for Wp C. Proceed by discussion between WP B & C partners, on what Cuba, DR, Guadeloupe, Martinique and Trinidad plan to do which might be relevant to this.
2. If Martinique and/or Trinidad conduct resistance trials, it would be useful for Workpackage B to design the work it to get information on disease progress (measure of incidence each week after planting, for at least 8 weeks, preferably ten) (C Langlais, P. Umaharan)
3. J. Holt to see if further epidemiological information can be obtained from Trinidad survey
4. C. Pavis, J. Holt & Raymond Bonhomme to discuss approaches to modelling the time series data (experiment 1 above). Some sort of explanatory models linking time series of whitefly number, whitefly infectivity, climatic and cropping data collected at Godet from 2001 to 2003 and from Vieux Habitant from 2002 to 2003.

5. Potential interaction between TYLCV and PYMV. J. Holt to discuss with P. Umaharan and C. Pavis. Compare progress curves for the two diseases with that expected if infection by one did not influence infection by the other.
6. Timing of disease 'take off' and resulting yields. Do we need to do some experiments or can we get this from the literature (John Colvin's India data?). Any project partners doing variety trials or testing possible IPM measures such as barriers to see if results of experiments can include yield measurements as well as disease progress curves (see Action Points 1 and 2 above).
7. T. Chancellor to take the UV-reflective much idea forward with more thorough literature review?
8. C. Pavis, T. Chancellor and J. Holt to decide if we take the barrier technique forward and how.

ANNEXES 1 - MEETINGS REPORTS 3

REPORT ON BETOCARIB WORKSHOP
HAVANNA, CUBA, 29 Nov. to 1 Dec. 2004

Workpackage A

This work package concerns i) the characterisation of begomoviruses affecting tomato, ii) the biotyping of vector populations of *Bemisia tabaci* in the Caribbean islands which were implicated in the project, iii) the incidence of begomoviruses, iv) the transmission biotest between begomoviruses and vectors and v) the identification of weeds infected with tomato begomoviruses. The results were supposed to be obtained at month 24. The principal results were obtained in time but for different reasons, some experiments are in standby at this stage of the Betocarib project.
During this meeting, the results obtained in 2004 were presented by the different partner attending the meeting (Partner 1: M. Peterschmitt, C. Urbino, and partner 6 : Y. Martinez). Then, we have had a discussion about the work which has not already been done.
Virus characterisation: the work is accomplished for all island except for the Dominican Republic for which it was not possible yet to analyse enough samples from isolated tomato production areas. This is due to some difficulties to extract DNA from collected samples in DR and send it to the partner 6 at Cuba. This will be discussed with the partner from the Dominican Republic when he will meet partner 6 from Cuba in the next months.
Whitefly characterisation: the B biotype was found to be dominant in the different island. The biotype A was found in Guadeloupe and Martinique but only in one sample collected in each island and not on tomato. Biotype A is therefore not expected to be involved in the epidemiology of the begomovirus diseases. The work is going on at Cuba (analysis of DNA sequences) and at Trinidad (cloning and sequences of DNA fragment). These partners are waiting for the funds to be able to finish their research.
Incidence of begomoviruses in tomato crop The work was accomplished in the different island for the different identified begomoviruses.

Transmission biotest from tomato to tomato: it has been done for all except Cuba and Trinidad because of some difficulties to adapt the protocol which was defined and also because of the lack of funds to buy the material. The different partner have discussed about new adaptations of the protocol and the tests will be done.

Identification of weeds, reservoir of tomato begomoviruses : No weeds were found to be infected with PYMV or PYMTV in Guadeloupe and Trinidad. TYLCV was found in Bean (Phaseeolus vulgaris) but this crop is very rare in Guadeloupe and is not expected to play a significant role in the important epidemics which is observed all over the island. TYLCV was found in a *Cucurbita pepo* plant in Cuba but the transmission to tomato has to be demonstrated.

Definition of new primer for the detection of begomoviruses from the old or the new world: This goal was added to the objectives of the project after its submission because of the possibility of the different partners to interact and exchange results on different begomoviruses. Until now, no interesting result was obtained; but we have to finish to test the proposed primers. Partner 1 at Montpellier will test the compatibility of the primers Rep 290/cpc405, and cpc534/Rep 219. The couples will be then tested by Partner 1 and 6.

A session was dedicated to the possibility to write **common publication** concerning the results obtained in WPA. According to the results now available, the following propositions were done:

- The diversity of begomoviruses affecting weeds in Guadeloupe and Cuba
- The incidence of TYLCV in Guadeloupe, Martinique and Cuba
- The biotyping of B. tabaci in Guadeloupe, Martinique, Dominican Republic Trinidad and Cuba. The insects from Cuba should be treated with the same tool as the insects of the other islands to allow the comparisons of the results. This work could be done at Montpellier by a student from Cuba in 2005

WORK PACKAGE B: Epidemiology and Modelling

Summary

The results of the epidemiological survey from Cuba and from Trinidad were presented and multivariate data analyses were presented. For Cuba, two datasets were analysed separately based

on data collected by IHLD and CENSA, respectively. Results were shown initially in the form of a series of contingency tables. In each case, these showed several significant associations between variables measured and disease incidence and/or severity. These significant variables tended to be linked and the linkages were examined further using principal components analysis. In the CENSA survey (which covered the eastern half of the country), ***high geminivirus disease incidence*** was associated with a combination of a greater abundance of previous hosts, greater host continuity and higher numbers of whiteflies.

Higher whitefly numbers were also associated with high disease incidence in the IHLD survey, as were later planting dates, the growing of susceptible varieties, and a greater distance to the edge of the plot. ***Low disease incidence*** in the CENSA survey was associated with better management practices, later observation dates, location (more westerly sites), higher altitude and (in contrast to the IHLD dataset) later planting. In the IHLD dataset, low disease incidence was associated with chemical and physical protection in the field and the nursery, sheltered cultivation and good management practices. Findings from the Trinidad survey were not as conclusive as those from the Cuba survey and further discussion is needed to clarify the precise nature of some of the variables. However, it was apparent that location affected the time it took for begomovirus disease saturation to be reached. The presence of non-hosts was linked to location and, by association, with the longer time to reach disease saturation.

The survey results from Guadeloupe and Cuba were then synthesised and a table of common significant associations between disease incidence and the recorded variables was drawn up. This was used as the basis for the identification of candidate components for the IPM packages to be tested (see report from Work Package C). Particular attention was paid to those variables that were negatively associated with disease incidence as these had the potential to be manipulated in disease management practices. The data obtained from such trials would also allow further validation of the crop model.

The crop model was described and the outputs from an analysis of the two 'barrier' experiments carried out in 2003 were presented. The outputs showed that barriers, used in combination with a yellow insecticide-treated strip inside the plots, were effective in reducing disease incidence in the season where disease pressure was low. However, when disease pressure was high (and in the absence of an insecticide-treated strip) the barriers did not reduce disease incidence. The presence of higher whitefly numbers in the barrier plots suggests that, under these conditions, the barriers prevented emigration and that some means of whitefly control within the plots is needed.

A detailed description of all four of the barrier trials conducted during 2003-04 was given. Barriers were effective in reducing begomovirus disease incidence during low incidence periods and the presence of an insectide-treated yellow strip appeared to enhance the effect.

Data were presented from an insecticide trial conducted in the field on the IHLD research station in Cuba. The results indicated that imidacloprid, as customarily applied by farmers in Cuba, was not effective in reducing whitefly numbers or disease incidence.

It was agreed that data from the Cuban survey would be re-analysed using the three geographical divisions (western, central and eastern) defined by IHLD and CENSA. The main output from the survey work would be a journal paper incorporating the datasets from Guadeloupe, Cuba and Trinidad. Further work on the crop model would be possible depending on the availability of data from additional disease management experiments proposed for Cuba (insecticide trial) and Martinique (intercropping, mulches, grafting).

Disease surveys

1. Cuba

Data were presented by Yamila Martinez on geminivirus disease incidence and severity and on whitefly abundance in the survey conducted in Cuba. The island was classified into three geographical regions:

- West: La Habana
- Central : Santi Spiritud and Villa Clara
- East: Holguin and Granma

The results showed that disease incidence was significantly greater ($p<0.05$) in the East (44.8%) than in the West (25.4%) or Central (15.8%) regions. Severity was also higher in the East than in the other two regions, but there was no difference in whitefly abundance among regions. The higher disease incidence and severity in the East was attributed, at least in part, to the presence of a greater proportion of susceptible varieties.

A more detailed assessment of findings from the epidemiological surveys conducted in Cuba and Trinidad were presented at the workshop by Tim Chancellor, following analyses conducted by John Holt. For each of the surveys, some simplification of the variables recorded was needed prior to analysis of the data. This was because in some cases the values recorded for particular variables were almost all the same. For certain other variables there were few instances of one of the three possible categories. For these latter variables, adjacent categories were combined into a single category. As a result, it was appropriate to examine the data with a series of contingency tables. The significant variables tended to be linked, so for example, the same field may be scored as having high previous hosts, high host continuity and high *Bemisia* abundance. These linkages were examined using principal components analysis (PCA) and fields in the Cuba survey were plotted by the first two principal component axes.

The results of the PCA plots from the ***CENSA survey*** showed that high disease incidence was associated with a combination more previous hosts, more continuity, and more *Bemisia*. In contrast, low incidence was associated with better management practices, later observation day, more 'westerly ness', higher altitude and later planting. It was not clear whether the low incidence in the west was associated with better management practices *per se* or with the other conditions prevailing at a different location. The other significant variables identified from the contingency tables had less clear effects. Generally higher temperatures (higher Tmax and higher Tmin) had some association with higher levels of disease, as did distance to the edge of the vegetable area.

It was unexpected that later planting was associated with low disease incidence. Further investigation indicated that planting date was very strongly positively associated with both observation day ($P=0.007$) and with management practices ($P<0.001$). This perhaps suggests that the other factors associated with lower disease at the more westerly locations outweigh any potential effect of later planted crops being more at risk.

In the ***IHLD survey***, there were many significant positive relationships between individual variables measured and disease incidence and severity. The strongest relationships in the data were between disease incidence and severity themselves ($Chi^2 = 154.22$, $P<0.001$) and between the disease variables and variety susceptibility ($Chi^2 = 96.09$ & 99.71, $P<0.001$). As with the CENSA data, principle component plots were made of the fields and of the eigen vectors of the variables. Higher incidence was linked to later planting dates, more susceptible varieties, a larger distance to the plot edge and larger numbers of *Bemisia*. Low incidence on the other hand was linked to chemical and physical protection in field and nursery, use of sheltered cultivation and good management practices.

Perhaps more unexpected, low incidence was also linked to the growing of summer crops, the presence of previous hosts and host continuity. Whilst these variables might be expected to increase begomovirus disease problems it appears that in this data set they occurred in conjunction with other variables which may have a counteracting effect of reducing incidence. It is possible that intensive and continuous production need not lead invariably lead to high disease levels provided that disease control measures are also in place.

2. Trinidad survey

The findings from the survey conducted by Pat Umaharan showed that *location* had a strong influence on the time it took for begomovirus disease saturation to be reached. The presence of non-hosts was linked to location and, by association, with the longer time to reach disease saturation. The results were not discussed in detail in view of the need to seek clarification on some of the variables recorded. It was agreed that this would be followed up with Pat Umaharan who was not able to attend the Workshop.

Epidemiological research in Cuba

Yamila Martinez presented the results from a study conducted on a farm at Guira de Melena. Observation plots of a commercial disease-tolerant hybrid variety (ARO 3019) and a susceptible

variety (Amalia) were planted. Sticky yellow traps were placed at three heights above the ground and whitefly numbers were counted at fifteen day intervals. Disease incidence (symptoms) and virus infection (HAN) were assessed every seven days. A significantly greater number of whiteflies were trapped at the lowest height above ground level. Climatic data were also collected and were used to develop predictive models of whitefly abundance and disease incidence. Following discussion of the preliminary findings it was suggested that, if possible, data should be collected over a longer period and that details of the modeling approach should be provided to assist interpretation of the results.

Jany presented the results of an experiment conducted in December 2003 to March 2004 on the IHLD research station in which the effect of imidacloprid on whitefly abundance and begomovirus incidence was assessed. The effect on applying imidacloprid in the nursery and in the field was examined through a field trial with four insecticide treatments arranged in a randomized complete block design. Plots contained 15 tomato plants and the treatments were:

1. No insecticide (susceptible control).
2. Gaucho 70 WS applied to tomato seedlings at a rate of 70g.kg of seed.
3. Confidor 350 SC applied at 35 days after sowing to plants in the field at a rate of 0.5 l/ha^{-1}.
4. Gaucho and Confidor (applied as described in treatments 2 and 3 above).

Disease incidence and severity and whitefly abundance were assessed at 30 and 60 days after sowing (DAS). Disease incidence was high at 30 DAS, ranging from 73 to 77% diseased plants, and there was no significant difference between treatments. Disease incidence at 60 DAS reached almost 100% in each of the treatments. Whitefly numbers were low and did not differ between treatments. The results indicated that imidacloprid, as customarily applied by farmers in Cuba, was not effective in reducing whitefly numbers or disease incidence. It was probable that the protective effect of the insecticide was no longer in evidence at the time the tomato plants were removed from the nursery and planted in the field (at ? DAS). Significant infection may have taken place during the period between transplanting and the application of Confidor at 35 DAS. However, it is not possible to establish this with certainty because the disease status of the tomato plants at the time of transplanting was not known.

Epidemiological research in Guadeloupe

Claudie Pavis presented data from (a) monitoring of whitefly abundance and infectivity at Godet, Grande Terre (b) four barrier experiments carried out in 2003-04 and (c) a field trial in which tomato was intercropped with coriander.

The monitoring data covered the period January 2003 to August 2004 and represent the continuation of a long term study that has continued for three years. Aerial trapping of whiteflies revealed that whitefly abundance was low during March and April when the first of two barrier experiments was conducted in each year. During the same period whitefly infectivity, as measured by the proportion of infected trap plants, was also low. These findings help to explain the relatively low disease incidence during the trials that were conducted in the early part of the year. By contrast, whitefly numbers and infectivity were relatively high between mid-June and mid-August. This may account for the higher disease incidence in the field trials carried out during this period.

Plants in tomato plots at the field station at Godet, were assessed for begomovirus disease symptoms and virus infection (PYMV and TYLCV) from planting until ten weeks after planting. PYMV predominated in 2003 and 2004 in spite of the fact that laboratory studies showed that it had a lower transmission efficiency that TYLCV. However, survey data collected from other areas in Guadeloupe and from Martinique showed that there was no clear pattern in the relative occurrence of PYMV and TYLCV. It is possible that the occurrence of the two viruses depends on factors such as cultural practices, variety and degree of intensification of tomato production. Further, as TYLCV is a relatively recent entrant to Guadeloupe and Martinique, the prevalence of the viruses is likely to be in a state of flux and this may contribute to the lack of clearly observable patterns.

As expected from the trapping and infectivity data, whitefly numbers and disease incidence were low in each of the two barrier experiments planted early in the year. In each case, the presence of a barrier around the plots resulted in significantly lower disease incidence. However, in the two barrier trials conducted when disease pressure and whitefly numbers were higher there was no significant difference in disease incidence .between barrier and control treatments. Interestingly, in the 2004 trial

whitefly numbers in the barrier plot were significantly higher than those in the control. It is hypothesized that this result was influenced by the absence of an insecticide-treated strip placed along the inside of the barrier. This strip was only used in the first (low disease incidence) trial in 2003. Thus, the presence of a whitefly trap inside the plots may be necessary to ensure sufficiently high mortality to reduce disease incidence. These results are discussed further below in the context of crop modelling.

A field experiment was carried out in Guadeloupe in which tomato was planted as an intercrop one month after coriander. Begomovirus disease incidence at five weeks after planting was significantly lower in tomato plants intercropped with coriander. However, there appeared to be strong competition between the tomato and coriander plants resulting in reduced growth of the tomato plants. The experiment was terminated early due to accidental damage caused to the plants by chemical application.

Crop modelling
Tim Chancellor described the 'Crop Model' that was developed by John Holt. The model simulates disease processes within a crop or a series of crops within a locality. The model is designed to describe disease processes in a biologically meaningful way, using parameters that have clear biological meaning. As a result, the model can be used to investigate the likely impact of management interventions to control whiteflies or disease incidence. For example, in plots protected by cloth barriers, immigration of whiteflies is reduced. Immigration rate is one of the model parameters and so the relationship between immigration rate and disease progress in the crop can be explored using the model.

Initial estimation of parameter values for the model has been done from information available from experimental work. Further estimation of these values has been done through a process of curve fitting. Data for curve-fitting were obtained from the field experiments to test the effect of cloth barriers in reducing whitefly infestation of the crop. Three pairs of disease progress curves have been generated from these experiments, together with corresponding data on the relative abundance of whiteflies in each member of the pair (a) disease incidence on tomatoes under low inoculum conditions, with and without barriers and *with a yellow insecticide strip* (b) disease incidence on tomatoes under high inoculum conditions, with and without barriers (c) separate curves from the plots without barriers on TYLCV and PYMV based on virus specific detection in a sample of plants.

The effect of the barrier treatment was examined by varying the model parameters that were likely to be affected by the treatment. Initially, only whitefly immigration was altered but a good fit to the data from the barrier treatment could not be obtained. However, when emigration (or loss rate) was also allowed to vary, a good fit to the barrier treatment data was obtained. The best fit to the data was obtained when immigration was reduced twelve-fold and emigration (or loss rate) was also reduced by about 25% since the yellow insecticide strips inside the barrier were expected to increase whitefly death rate. The model output suggests that overall whitefly loss (emigration plus death) within the barrier plots was actually less than in the control plots. This may be due, in part, to the effect on whitefly mortality of the death of natural enemies on the insecticide-treated strips. Therefore, the effect of the barriers on virus disease infection appears to be the net result of both positive and negative influences. A key management issue is to consider whether the technique can be modified to reduce the negative effects.

Linkages with Work Package C on Integrated Pest Management
Tim Chancellor and Claudie Pavis gave a short presentation summarising how the outputs from Work Package B should contribute to Work Package C on Integrated Pest Management. In short, the epidemiological surveys were used to identify key components of begomovirus epidemics. Variables that were associated with reduced disease or whitefly incidence, such as physical protection and barriers, were assessed to assess the potential for utilising them for management purposes (see Table). An additional approach was to use the crop model to simulate (a) begomovirus disease progress in the tomato crop and (b) the effect on disease progress of different control measures and their combinations.

Support was then given to the process led by Olimpia Gomez and Christian Langlais in which potential management interventions were identified and IPM packages developed for evaluation (see Workshop report on Work Package C). It was stressed that certain experiments might serve the dual purpose of

assessing the effectiveness of component management technologies and validating the crop model. Guidance from John Holt was passed on to participants in the workshop session. The main point was that disease progress curves and measures of whitefly abundance should be obtained for at least two treatments at the same location. This is because it is expected that only one or two of the model parameters are likely to vary between the two treatments and so the model outputs are more useful. Disease management experiments proposed for Cuba (insecticide trial) and Martinique (intercropping, mulches, grafting) could potentially be used for crop modelling work. Action: *Olimpia Gomez and Christian Langlais to discuss possibilities with John Holt.*

Table Variables influencing geminivirus disease incidence on tomatoes in Guadeloupe and Cuba, based on survey data.

Guadeloupe	***Cuba***	***Low/high disease***	***Common factor***
Chemical protection	Chemical protection	Low	Yes
Physical protection	Physical protection	Low	Yes
Barriers	Sheltered cultivation	Low	(No)[3]
Management practices	Management practices	Low	Yes
Non-hosts (BT)[1]		Low	No
	Distance to edge (increasing)	Low	No
Hosts (GT)[2]	Previous hosts/host continuity	High	(No)[3]
Virus sources		High	No
	Susceptible variety	High	No

[1]Basse Terre
[2]Grande Terre
[3]The variables are nevertheless closely linked

WP C PLANT SCREENING

This work package is dedicated to (i) define different strategies of virus control based on the use of tolerant/resistant cultivars and the appropriate crop management according to WPB results, and to (ii) IPM evaluation in different agronomic and socio-economic conditions.

Olimpia presented the results obtained from TASK T03.01 and TASK T03.02- Screening varieties for resistance to the identified begomovirus and to climatic adaptation. At the beginning she talked about the two main major constraints that were identified in the participating countries (Martinique, Trinidad and Tobago, Dominican Republic and Cuba) so resistance to begomoviruses and to *Ralstonia solanacearum* are needed in some places in Martinique and in isolated environments in Trinidad. Besides, climatic adaptation is needed in all the countries as well as local market adaptation. The antecedents of this work were also exposed which let her furnish the criterion to select the origin (mainly from Israel and Cuba) of the tomato samples (cultivars) that must be sent to the other partners.

12 trials were carried out in the project (3 in Martinique, 3 in Trinidad and 6 in Cuba) which involved 349 cultivars or entries. Another trial has been developing in Trinidad now, and they just finished a new one in Martinique in which natural infection was not high so there weren't important differences between cultivars, besides a cultivar validation has been developing in Cuba now.

The results obtained showed that it was possible to identify cultivars tolerant to a number of begomoviruses reported in the countries (Martinique, Trinidad and Cuba); that it was possible to select cultivars with general adaptability and acceptability in the environments tested and that a strong genotype x environment effect exists in some countries as Trinidad and Cuba which let recommend cultivars for the dry season and for the wet one.

Data from these trials concerning to yield and virus incidence and severity were bring as well as the general characteristics of the promising cultivars tested.

According to the obtained results it is possible to build an IPM package during the workshop that will be carrying out in this third meeting in order to evaluate it in different agronomic and socio-economic conditions next year.

Other activities made by partner 7 such as publications and the scientific meetings were also reported.

After Olimpia´s presentation, Christian presented the results of the last trial carried out in Martinique which showed no important results because of the lack of infectation. He also explained different situations that may be found in Martinique according to the two major constraints that exist there, it means begomovirus and Ralstonia, and refers the importance of some cultural practices identified and well studied for him, such as the use of isolated nurseries and grafting combined with a useful cultivar as an agronomic solution since not a resistant or tolerant cultivar to both constraints has been reported until now.

ANNEXE 2 - TECHNICAL FACSHEETS 1

The objective is to evaluate the incidence of begomovirus (es) in tomato in each island.

Before doing that, each country must have evaluate how many begomovirus infect tomato as described in the first meeting report (point 2 page 1)

1) If only one, then the incidence can be evaluated with **general tools** as ELISA or hybridisation with generic probe. Visual symptoms are not enough because if other viruses occur (as in Trinidad) it is not easy to estimate the real incidence of begomoviruses.

2) if more than one, then **specific tools** should be used to estimate the relative incidence of each virus : I mean if you look at 100 plants how many are infected with begomovirus (in general) , how many with begomoN°1 and how many with BegomoN°2 etc. Even if one is supposed to be minor, the diagnostic must be done for it (example PYMV or other begomoviruses in Dominican republic)

How to conduct the survey in the two cases

1) **The different location must be identified (different ecosystem defined as in point 3.2).**
2) **40 samples must be collected in each field.**
3) **Around 10 fields must be surveyed per location.**

this means 40x 10 = 400 samples per location.

Each sample must be treated with:

- in case 1 :a generic probe or ELISA
- in case 2 : generic probe and specific probe for each begomovirus identified in the country.

4) When should we collect the samples?
Once at the beginning of the growing season, and once at the end of it. This will allow verifying if the relative incidence of each begomovirus is the same at the beginning and at the end of the tomato season.

5) How should we select the fields?
We should collect samples in fields at the same age (5 to 7 weeks after plantation). This because it is difficult to compare the level of contamination in fields of different ages. At this stage (5-7 weeks after plantation), the different viruses occurring in the region must have infected the plants. After this stage, it will be difficult to distinguish secondary contamination from the first ones.

6) How should we collect the samples?
The samples must be taken from a random selection of plants within the field irrespective of their symptom status. If only plants with symptoms are selected this will lead to a biased estimate of the relative incidence of the viruses. Thus, if only symptomatic plants are selected, the more virulent virus and or virus combination will be over-represented. In addition, virus may be present in plants that have not (yet) expressed symptoms.

7) Which part of the samples

- for ELISA, the best that we have test in Guadeloupe is the upper part of the stem just under the apex : 2-3 cm of the stem located under and upper the youngest adult leave with this leaf
- For molecular hybridisation, the apex is the best. We use the protocol in annex. It is the same that is described by Potter (Dellaporta modified by heating the samples) but we add a precipitation at the end to concentrate the virus. **Information about this protocol is given in another document that will be add on the web site of betocarib soon**

****Particular to Cuba
They said that they can not go twice in the fields for practical reasons so in part 4) they should define at which period there is the great risk of contamination with begomovirus and then make the survey 5-7 weeks after the beginning of this period. I don't know if they can find fields planted 5-7 weeks before and continue with 5)

ANNEXE 2 - TECHNICAL FACSHEETS 2

Use of hybridisation to detect begomoviruses, using the protocol issued from Jamie Lee Potter

Yamila gave this protocol to the different participant during the first meeting session in Guadeloupe (Appendix 2-3 and 2-9). I can fax it again if you need. We have tested it in Guadeloupe for PYMV with success. The kit from Amersham is very easy to use. A Comparison with Dig High prime procedure from Roche diagnostic system (Boehringher) was done by Jamie Lynne Potter (University of Wisconsin, Madison). The table is given in this file.

Obtaining of probe

We use a fragment of 420 pb (part of IR and 5'ter of CP) amplified with primers MP16-MP82
This DNA was labelled with the AlkPhos Direct labelling from Amersham (Ref RPN 3690)

DNA extraction

DNA was extracted from tomato samples using the protocol modified from Dellaporta's protocol by heating the sample (appendix 2-3). Better results were obtained in chemiluminescent revelation if DNA obtained in the protocol was concentrate by precipitation with 500 µl isopropanol and the pellet dilute in 50 µL of water.

Dot blot

3µL of such DNA preparation is laid on the membrane Nylon N+ after denaturation with NaOH and EDTA and heating 10 min at 100°C
DNA is fixed on the membrane by heating 2 hours at 80°C

Revelation

1-hour exposition in the substrate
2-hours can give more coloured spot but 4 hours is not better than 2 hours
Viruses were detected in positive control until dilution 1/100

After the first use, the efficiency of the probe decrease but the probe can be use 3 times for diagnosis without problem.

This table is issued from the thesis of Jamie Lynne Potter and the University of wisconsin, Madison

Component	AlkPhos Direct	Dig High prime
Level of background	low	moderate
DNA preparation for probe	1 PCR reaction	5-10 PCR reaction
DNA concentration for probe	100ng	1µg
Labelling reaction time	45 min	1h to overnight
Probe storage time	6 month	1year
Solution storage time	4-6 month	4-6 month
Solution storage temperature	4 or –20°C	4 or –20°C ot room temp
Hybridization time	4h to overnight	6h to overnight
Wash time	60 min	40 min
Immunological/Detection time	2-5 min	1.5 hour
Signal emission detection time	5-30 min	5-30 min
Film exposure	4h	4h
Reuse of the probe (cica's experience)	*less than 4 time*	*More than 5 times*

ANNEXE 2 - TECHNICAL FACSHEETS 3

BETOCARIB - BEgomovirus diseases management for sustainable production of TOmato in the CARIBbean CIRAD - IIHLD - INRA - ISA - NRI - UWI

INRA Antilles-Guyane
Unité de Recherches en Productions Végétales
Programme *Bemisia*/begomovirus

Procedure BETOCARIB-A-Tr PYMV TT Gpe 2002

Written by C. Pavis, 11 April 2002 Version n°01
Validated by C. Urbino, 11 April 2002

Title: Determination of the transmission rate of the PYMV from tomato to tomato by *Bemisia tabaci* .

Participants:

Objective: in order to know the efficiency of *Bemisia tabaci* as vector of begomovirus, it is necessary to determine at what rate the insects are able to transmit the disease to tomato, in the general case where the vector breeds on tomato.

Method: to infect tomato plants in controlled conditions, with insects, which acquired the virus on, infected tomato plants in insect-proof cages, during 48 hours. Batches of different numbers of insects are used to infect tomato plants, during 48 hours. The symptoms are observed and then ELISA tests are carried out.

Experimentation:

Insect rearing

The insects are reared in a "Maxiserre", in climatic rooms. About 300 newly emerged insects are disposed in the Maxiserre containing 2 cabbage plants and they are removed after 5 days (**it is important to remove all the insects present in the cage and on the plants**). With temperature of 25°C, the larval development is about 25-28 days. With an initial infestation of 300 insects, 1500 adults are expected at the next generation. The peak of emergence of insects is about 4 days after the first emergences.

Acquisition of virus by insects

At the emergence peak, all the insects are collected, using the mouth vaccum-cleaner. **They have to be gently collected**, to avoid stress and mortality. When the glass tube contains about 100 adults, it can be transferred in another cage, containing 2 infected tomato plants (see procedure for grafting, or agroinoculation). The tube has to be inserted vertically, without lid, in the ground of the tomato plant. The insects will then go out of the tube. These operations have to be repeated until all the insects are collected from cabbage cage.

The tomato pots have to be put on plastic plates, and **irrigated in a way to avoid free water on the plate** (free water causes mortality in adults, when they land on the water).

- The acquisition period is 48 hours.

Plants for inoculation

Tomato plants, variety Caraïbo, with 2 true leaves (one week for the seedlings, one week in a 0.5 l container). Plants have to be grown in an insect-proff greenhouse or insect-proof cage.

Inoculation

After 48 hours of acquisition, **females have to be sorted**. They are bigger and fatter than the males. For training to sort females, you have to collect it with the mouth vaccum-cleaner (for example, collect batches of 5 suspected females, then you have to control the sex under the binocular). When you are good for sorting, you can begin the experiment.

Batches of 3, 10 and 25 females have to be prepared. Always collect 3, then 10, then 25, then 3, then 10, then 25 insects etc..., in order to carry out the same number of replicates for 3, 10, 25 females for

inoculation, the same day. When the tubes are ready, you can control if there is no males, under the binocular.

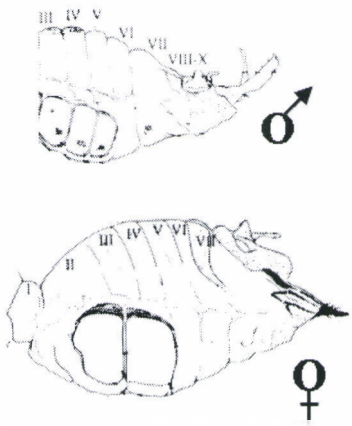


Figure: lateral view of the abdomen.

When the tubes are ready, the inoculation is carried out.
A tube is inserted vertically, without the lid, in the ground of the topato pot. Just after that, the plant is covered with a transparent aerated plastic tube (about 6 cm diameter, 8 cm high). A label is placed on the pot, indicating the date, the tested virus, the number of insects for inoculation and the number of repicate. Ex. : 21-05-02/PYMV/10-3).

Number of replicates:
50 plants infected with 3 females,
50 plants infected with 10 females,
50 plants infected with 25 females.

The inoculation has to be carried out far away from the insect-proof greenhouses, to avoid their infestation.

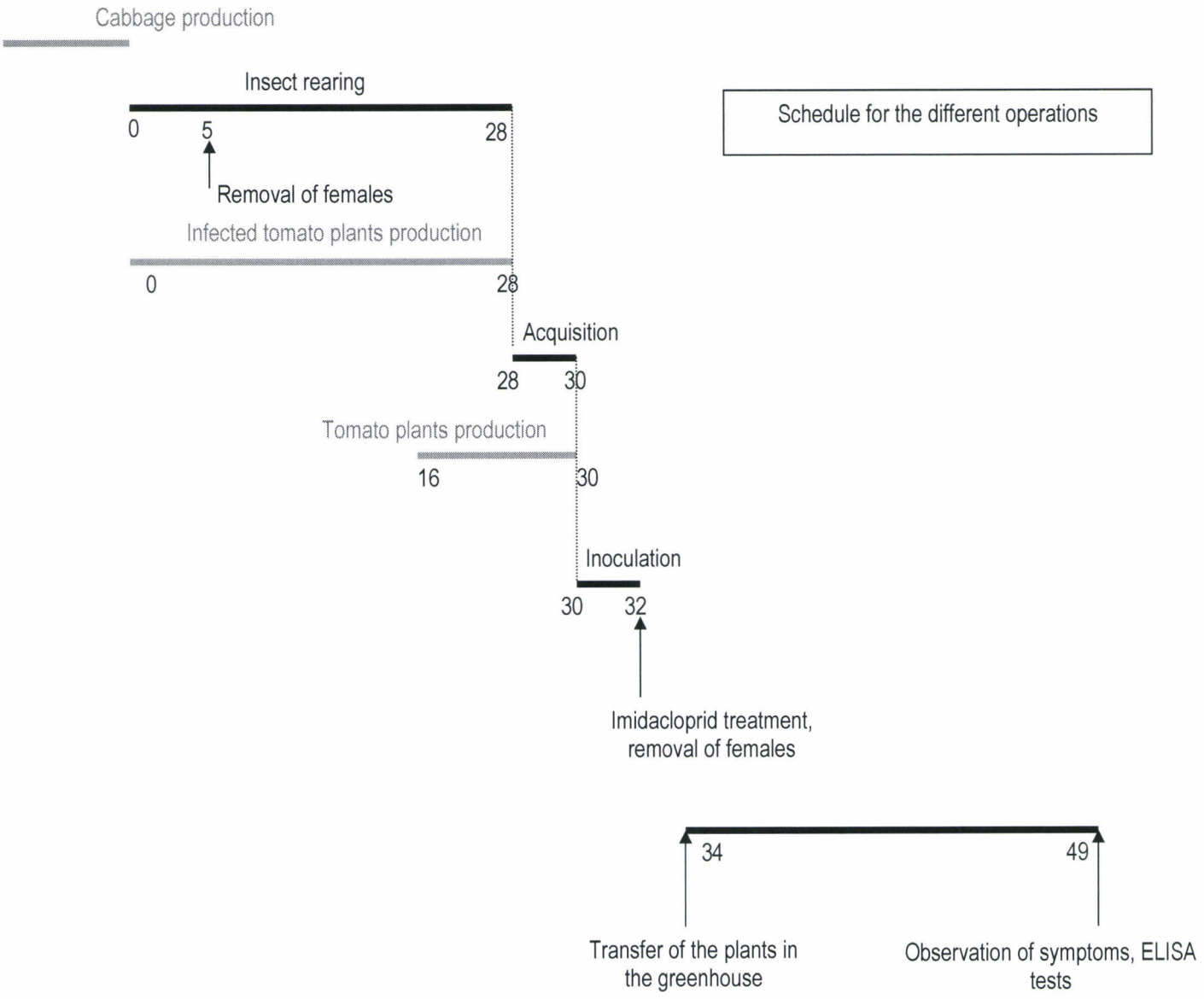
- 1 day after inoculation, you have to control if the females are on the leaves, or if they are dead. If they are not well fixed on some plants, you have to discard these plants.
- 48 hours after inoculation, the plants are treated with Imidacloprid (3 ml/l), the cages are removed and the females collected with the mouth vaccum-cleaner.
- 4 days after inoculation, the absence of adults on the leaves is controlled and the plants are taken to the insect-proof greenhouse.

Observations

- 19 days after inoculation: observation of symptoms and ELISA tests if no characteristic symptoms are observed.

Schedule
J-2 (acquisition): ?-?-2002
J0 (inoculation): ?-?-2002
J+2 (stop inoculation): ?-?-2002
J+19 (observation of symptoms/ELISA): 5-02-2001

Climatic conditions
In order to standardize the insect rearing, the acquisition and inoculation efficiency, we have to work with the same range of temperature: between 25 and 28°C. The relative humidity is between 80 and 100%. If possible, we have to recod temperature and humidity with a thermohygrographe, or with a data loger.



Result sheet on *PC Claudie : C:\Mes Documents\Claudie\BemGem\0-Manips\BETOCARIB\A***GpeTr PYMV T-T 2002-01**

ANNEXE 2 - TECHNICAL FACSHEETS 4

BETOCARIB - BEgomovirus diseases management for sustainable production of TOmato in the CARIBbean CIRAD - IIHLD - INRA - ISA - NRI - UWI

INRA Antilles-Guyane
Unité de Recherches en Productions Végétales
Programme *Bemisia*/begomovirus

Procedure BETOCARIB-B-Epid-survey-Gpe 2002

Written by : C. Marchal, 15 May 2002
version n° 03
Validated by : C. Pavis and J. Holt, 22 May 2002

Title : Epidemiological survey of begomovirus diseases in tomato fields

Participants : Cécile Marchal
Albert Huc
Roseline Gotin
Others occasionnally
In collaboration with techniciansfrom SAFER and Chambre d'Agriculture.

Objective: To determine the key factors associated with epidemics of a begomovirus disease (Potato Yellow Mosaic-Virus) transmitted by the whitefly *Bemisia tabaci* in tomato. The data will be used to build an ecosystem model.

Method: Survey 100 tomato plots in each selected growing zone (Grande Terre and southern Basse Terre in Guadeloupe). The survey form is detailed below. The corresponding file is BETOCARIB_B_Epidemio-datasheet 2002.

Procedure:

1) Make an appointment with the grower (usually call the day before).

2) Material: Bring a detailed map of the region and a GPS to localize the fields, a cell phone, writing boards, waterproof pens and datasheets (Computer Coccinelle, file BETOCARIB_B_Epidemio-datasheet 2002).

3) When at the field,
- first interview the grower (when possible ; otherwise call him later to get the information),
- then fill in the general description of the plot,
- and finally score the disease incidence and evaluate the whitefly population.
  Depending on the plot size, decide of the distance (number of steps) between successive observations in order to describe at least 40 plants. When there are more than 1500 plants in the field, sample around 100 of them.

  To take the prevailing wind into account, score separately the 2 upwind and downwind edging rows and then others in between.

See the annotated survey form below.

4) Back at the office, determine the scores and enter these on the spreadsheet (the records file is BETOCARIB_B_Epid-survey-records_Gpe 2002).

5) Send the records file to John Holt and Tim Chancellor (e-mail addresses: J.Holt@greenwich.ac.uk; t.c.b.chancellor@gre.ac.uk) for analysis. In case of any doubt about any of the procedures contact John, Tim, Cécile or Claudie.

Data recording

Datasheet, results and connected information are recorded both in the lab notebook « Epidémiologie PYMV-Bemisia » (URPV 02 BB EPID), and in the Coccinelle computer (files BETOCARIB_B_Epidemio-datasheet 2002, BETOCARIB_B_Epid-survey-records_Gpe 2002.

Schedule :

The survey began in Guadeloupe on the 26 March 2002.
3 field trips a week are planned, enabling around 15 fields to be surveyed per week.
We expect to complete the survey by the end of June (or mid-July).

Detailed procedure: see the following data sheet, which is annoted with comments in bold font.

BETOCARIB_B_Epidemio-datasheet 2002, page 1

Observer : Date : Time :

Place (town): Plot :**location name + grower initial + n°**

Weather : **specify the wind speed (roughly) ; rain**

GENERAL DESCRIPTION OF THE PLOT

Geographic position (GPS + map) : **locate the plot on a map photocopy**

Topography

flat (1)	Valley bottom (2)	slope N (3)	Slope E (4)	slope S (5)	slope W (6)

Estimated age or growth stage : **has to be between the beginning of flowering and full production**

Beginning of flowering	Beginning of fructification	Beginning of harvesting	Full production

general scale: 1=low; 2 = medium; 3=high

Size and edge	-small <0.5ha -< 100 m	- 0.5-2ha - 100-200 m	-large >2ha -> 200 m	Note (1-3)
Size of the vegetable growing area				
Distance to the upwind edge				

Upwind edge : **describe briefly**

Barrier (1-3)= estimate the barrier quality of that edge (1 = poor whitefly barrier; 3 = good whitefly barrier)

Whitefly hosts (1=none; 2= some ; 3= abundant) Non-hosts « crops »

tomato	cabbage	Cucurbits	euphorbia	**overall**	forest	pasture	Cane or maize	«Buffer » zone	Other vegetable	**overall**
1 2 3	1 2 3	1 2 3	1 2 3	**=maxi-mum**	1 2 3	1 2 3	1 2 3	1 2 3	1 2 3	**=maxi-mum**

« buffer » ? : plowed water road buildings other

look for specified weeds inside and around the plot

Virus sources (1=none; 2=some ; 3= abundant)

	Tomato			Sida			Rhynchosia			overall
presence	1	2	3	1	2	3	1	2	3	**See the scoring scale below**
symptoms										

Scoring scale: - if everything scores 1: overall is 1
- as soon as one criterion reaches 3: overall is 3
- if presence is 2 without symptoms: overall is 2
- if presence is 2 with symptoms 2 or 3: overall is 3

Crop management :
NB :« plastic » refers to plastic covered tunnels ; « net » refers to insect-proof netting

Barrier crop rows		Protection (plastic, net)		Drip irrigation		mulch		weeding		Plants health	
yes	no	Yes	no	yes	no	yes	no	-	+	-	+

Physical protection : *(1-3)*

« good practices » score : (1-3)

scoring scales :

If none is positive :1 (for both scores)	
If greenhouse of plastic only or barrier rows are poorly developed: 2	(mulch was considered optional, and weeding and health as the most important parameters) If weeding and/or health are very poor : 1
If the greenhouse is entirely closed (by plastic or glass or insect-proof net) or there are both plastic and barrier rows or efficient barrier rows : 3	If any of the 3 main parameters is negative: 2
	If the main 3 are positive: 3

BETOCARIB_B_Epidemio-datasheet 2002, page 2

Date : Plot :

WHITEFLIES AND DISEASE

Incidence and severity (severity notes) (scoring 1 plant every steps) **depending on the plot size**

	upwind	Within the plot	downwind	total
1= no symptoms				
2 = mild symptoms **Curling, mosaic**				
3 = severe **Severe mosaic and curling, stunting**				
incidence	**=(2+3) / total**			
severity	**=3/(2+3)**			

Number of whiteflies observe 3 leaves on the plants scored for incidence and look for adults

	upwind	Within the plot	downwind	total
1 = none	*N_1 = number of plants in this category*			
N_1				
2 = some (<5 adults/plant)				
N_2				
3= moderate (5-10)				
N_3				
4 = many (>10)				
N_4				
Mean abundance score				

The mean abundance score as calculated below is based on the approximate numeric equivalents of scores

Mean = 1 + Ln[(2.7 N_2 + 7.4 N_3 + 20.1 N_4) / (N_1 + N_2 + N_3 + N_4)]

BETOCARIB_B_Epidemio-datasheet 2002, page 3

interviewer : Date : Plot :

PRODUCER INTERVIEW

Name: directly /
phone

Planting and cultivation details :
Variety :
planting date:
plantlets age at planting : (⇒age of observed plants =)
Surface and/or nb of plants :

Insecticides use :

yes	no	Confidor	Mixed ?	dose	frequency	Spray / drip	At planting	After ?

Field chemical protection score (1-3) : **efficiency evaluation**

Plot « history » : **what was that plot planted to before, and what was around it at plantation**
⇒ *previous potential hosts (1-3) :*

Tomato growing period :

sept	oct	nov	dec	jan	feb	march	april	may	june	july	august

« host continuity » score (1-3) : **1= long host-free period ; 3= year-round growing**

Nursery details :
Where ? :

Close to tomato fields	At home (ie far from fields ?)	Bought from a supplier
		Go and visit him !

Protection :

none	net	Plastic, greenhouse	other (?)

Nursery physical protection score (1-3) : **combining Where ? and Protection**

Scoring scale : - if « close to field » and no protection : 1

- if « close to field » and « plastic » or « far from fields » and no protection : 2
- If there is an insect proof net or « far from fields » and « plastic » : 3

insecticides use:

yes	no	Confidor / Plenum	other	Mixed ?	dose	frequency	Spray / drip

Nursery chemical protection score (1-3) : **efficiency evaluation**

ANNEXE 3 – VISITS 1

Report of Trinidad & Tobago visit
Yamila MARTINEZ

From February 8 to 14 , 2004, the Dr. Y. Martínez visited to the laboratory of the Dr P H Umaharam, in the West Indian University, how partner 5 of the project BETOCARIB, with the objective to compliment the activities of the project and know and update the results and aspects discussed during the meeting conducted at the end of 2003. During days 9 and 10 we worked in discussing the results obtained and presented by each partner of the project, Dr Martinez presented the results obtained by partner CENSA.

The protocols for DNA extraction in whiteflies and weed were discussed. The group worked in the discussion of the protocols of RAPD-PCR for the genomic characterization of whiteflies population and analysed the results obtained by the student of PhD of the Dr Umaharam.

We have also visited tomato fields in North and South region of Aranguez like representative of different ecosystems. We could also observe differences in the symptoms in tomato plant and in the systems and extension of the crops with respect to Cuba. We observed the small and dispersed areas of culture of tomato with poor agricultural practices. In the afternoon Dr Martinez was received by Professor Charles R. McDavid (PhD), Dean of Agriculture and Natural Science Faculty, to aspects of the project and future collaborations.

During this stay I learnt on the techniques IC-PCR developed by this group for the detection of begomovirus from plants extracts clarified and the comparison with standard procedures. Even though the generic primers did not arrive in time to be tested during the visit, we worked in the adjustment of the conditions of the PCR. The visit was fruitful because it allowed the update of the work which was executed by the different partners of the project with UWI, it allowed to know the production system to tomato in the country and to exchange knowledge on different techniques for the diagnosis in begomoviruses. During the visit the Dr. Umaharam and his work group gave us an excellent attention and hospitality.

Del 8 al 14 de Febrero del 2004, realice una visita al laboratorio del Dr. P H Umaharam , en la Universidad West Indian, partner 5 del proyecto BETOCARIB, con el objetivo de cumplimentar las actividades comprometidas como parte del proyecto y para la actualización de los resultados y aspectos discutidos durante la reunión celebrada.
Durante los días 9 y 10 trabajamos en discutir los resultados obtenidos y presentados por cada uno de los partner del proyecto, así mismo la Dr. Martínez presentó los resultados del 2003 obtenidos por el partner CENSA.
Se discutieron los protocolos de extracción de ADN de mosca blanca, así como de ADN a partir de malezas. Trabajamos en la discusión de los protocolos de RAPD-PCR para la genotipificación de mosca blanca y analizamos los resultados obtenidos por la estudiante de PhD del Dr. Umaharam.
Visitamos campos de tomate de la zona norte y sur de Aranjuez como representativa de diferentes ecosistemas. Pudimos observar diferencias en las sitomatologías presentadas y también en los sistemas y extensión del cultivos con respectos a los de Cuba. Llamó considerablemente la atención las pequeñas y dispersas áreas de cultivo de tomate y en algunas con pobre prácticas de manejo.
En la tarde fui recibida por el Profesor Charles R. McDavid (PhD), Decano de la Facultad de Agricultura y Ciencias Naturales, con el cual se sostuvo una larga conversación relacionada con aspectos del proyecto y de futuras colaboraciones.
Durante esta estancia recibí todo los conocimientos sobre las técnicas de IC-PCR desarrollada por este grupo para la detección de begomovirus a partir de extractos clarificados de plantas y su comparación con los procedimientos estándares.

Lamentablemente aún cuando los cebadores genéricos diseñados no llegaron en tiempo para ser probados durante la visita, trabajamos en el ajuste de las condiciones de la PCR.
La visita fue fructífera pues permitió en primer lugar la actualización del trabajo que ejecutan los diferentes partner del proyecto y en particular de UWI, permitió además conocer el sistema de producción de tomate del país e intercambiar conocimientos en diferentes técnicas para el diagnóstico de begomovirus.

ANNEXE 3 – VISITS 2

Visit to Cuba, March 2003, T Chancellor & J Holt (Partner 2)

SUMMARY

John Holt and Tim Chancellor made a one-week visit to Cuba on 15-21 March 2003. The visit was organised and hosted by Olimpia Gomez (IIHLD) and Yamila Martinez (CENSA). A series of field trips were made to tomato growing areas, including one that had been included in the epidemiological survey. Plans were prepared to survey the remaining fields and minor changes were made to the sampling protocol. A template for data entry was agreed, based on the spreadsheet used for the Guadeloupe survey. A plan was also made to try to access historical data from the Plant Protection Service on TYLCV incidence in Havana province. In addition, an insecticide trial to evaluate disease vector control options was designed and it is proposed that this will be conducted on the IIHLD experimental farm in 2003-04. John Holt and Tim Chancellor gave presentations to an audience of researchers from CENSA, IIHLD and associated institutions. The visit was extremely useful and the excellent organisation and kind hospitality were very much appreciated.

BACKGROUND

Epidemiological surveys are being conducted in Work Package B in order to provide the basis for the development of an ecosystem model and to identify potential interventions for the management of tomato geminiviruses. The survey in Guadeloupe was conducted first and preliminary data analyses have now been completed. These showed a strong association between good crop management practices (comprising irrigation, weeding and general quality of the plants) and reduced disease incidence at both Basse Terre and Grande Terre. In order to build on the experience gained from the Guadeloupe survey, it was agreed that John Holt and Tim Chancellor would visit Cuba in early 2003. This would allow them to familiarise themselves with the crop production systems in the country and to review the ongoing epidemiological survey with colleagues from IIHLD and CENSA. A further objective of the visit was to strengthen the links between work packages B and C and examine the possibility of designing experimental studies to address disease management issues.

VISITS TO IIHLD AND CENSA

Visits were made to IIHLD (17 and 20 March) and to CENSA (19 March). We were briefed on the activities of the respective institutes and given a tour of their facilities. At CENSA, we gave two Powerpoint presentations to an invited audience of researchers. Tim Chancellor described recent studies on the epidemiology and management of key virus diseases of two crops of interest to Cuba; namely, rice and groundnut. John Holt summarised the findings of the epidemiological survey conducted in Guadeloupe, comparing the results from Basse Terre and Grande Terre.

A series of meetings were held at which we discussed:
- tomato production systems in Cuba
- protocols for the epidemiological survey in Cuba
- future work on TYLCV management

These issues are considered in the following sections.

Varietal resistance

Israeli hybrids mostly have good tolerance to TYLCV and have been selected at high temperatures when virus titres are highest. However, as these hybrids were developed from selections grown in a dry climate they have little resistance to fungal pathogens such as alternaria and blight. These diseases are problems in summer tomato production in Cuba. The Cuban variety Vyta, developed at IIHLD in collaboration with INRA at Avignon, is well adapted to Cuban growing conditions and has good resistance to TYLC. A disadvantage is that fruit size is smaller than that preferred by most consumers in Cuba.

At present, Israeli hybrids are grown on approximately 1000 ha in Cuba. Vyta is grown on *c.* 6,000 ha, with the remaining 17,000 ha down to susceptible varieties. Hybrid seed is very expensive (up to $1,000 per kg). IIHLD aims to produce hybrids and is keen to utilise marker-aided selection in its breeding programme.
Some varieties developed by IIHLD as early as two decades ago are still widely grown today as they have good climatic adaptation and large fruit size. Their release preceded the emergence of the begomovirus problem, and they are susceptible to these diseases. Nevertheless, these varieties can still be grown successfully in some locations, particularly when planted early in the growing season.
Other pest problems include root knot nematodes, *Meloidogyne* spp., which may develop with continuous cultivation. Rotation with a non-host such as lettuce reduces nematode populations. Unlike in Guadeloupe, bacterial blight is not an important problem in Cuba. Consequently, new breeding lines are not selected for resistance to this disease.

Climatic factors

During the winter (November to March), a succession of cold fronts arrives in Cuba. Thus, sudden drops in temperature and equally rapid rises occur. The temperature conditions fruit set in tomatoes at 5-9 days before anthesis. Fruit set is favoured by low temperatures and is poor in the hot, wet season. Thus, tomatoes need to be well adapted to the climatic conditions in Cuba in order to obtain optimum yields. The optimum period to plant tomatoes is from October to December and yields reduce significantly when planting is done from January onwards. Israeli hybrids are not well adapted to the variable temperatures that occur in Cuba, especially the high humidity. The average number of cold fronts occurring over the winter growing season (Nov. to March) is *c.* 20. Tomato yields are related to the number of fronts arriving during this period.

Sheltered cultivation

Large closed installations imported from Israel (5,000m^2) are better for use during the winter as temperatures become too high in the summer, especially at the centre of the enclosures. An open construction is needed in the summer period. A new open rectangular design (10-12 x 40-50m) has been developed and promoted and examples of these were seen at IIHLD. The structures are produced through a joint venture between Spanish and Cuban companies. These houses have a large permanent ventilation opening at the roof apex and the sides of the house are made of netting rather than plastic. Drip irrigation is used in these houses. Currently, *c.* 100 ha of tomatoes are grown in sheltered systems in Cuba. The Ministry of Agriculture is keen to promote the use of sheltered cultivation to increase tomato production in the winter and so help to stabilize tomato availability over the year.

REGULATORY MEASURES

Certain restrictions imposed on planting dates, with a cut-off date of 15 January imposed for most varieties. However, new regulation permits the planting of the TYLCV-resistant cv Vyta up to 15 March.

Agronomic practices

- Most farmers use irrigation; sprinklers are common in larger-scale production.
- Mulching not commonly practised and barriers of non-whitefly hosts such as maize around tomato fields are scarcely used, as a deliberate measure against begomoviruses

Tomato plants grown in individual plastic containers (Speedlings) are now commonly used (mainly for hybrid varieties), as these establish rapidly in the field after transplanting.

- Imidacloprid is applied either as a seed dressing (Gaucho) and/or at transplanting (Confidor). Gaucho is a wettable powder formulation applied at a rate 70 g per 1 Kg of tomato seed. This is done immediately before sowing. The effect of the insecticide is said to last for 30 days.

Field visits

Trips to three field sites were made during the visit. A planned trip to a fourth site in Pinar del Rio Province was abandoned because heavy rain had caused severe damage to the tomato crop and the farmers harvested early to try to avoid further losses.

On 18 March, visits were made to two farms in Guira de Melena district in Havanna Province. The first grower, Mr Raphael Baez, was a progressive farmer who has good links with agricultural research institutions. He also has a weather station on his farm. He was currently hosting separate trials on sweetpotato and tomato, respectively. The tomato trial was being conducted in association with CENSA and involved two varieties: HA 3019, a new Israeli hybrid that is tolerant to TYLCV and Amalia, a locally adapted variety which is susceptible to TYLCV. A single block of each variety was planted on 13 December 2002 and no insecticide was applied. TYLCV symptoms (incidence and severity) were assessed weekly. Weekly counts of whiteflies on the plants were made. Sticky yellow traps were also placed in the plots and whitefly numbers on these were assessed every fifteen days. The data are currently being collated and analysed. We observed late infection in the plot of Amalia. No disease was seen on CV HA 3019.

Action points: *Yamila and colleagues to provide a summary of the whitefly abundance data in the trial.*

Another progressive grower who produced vegetables under contract for the Havanna tourist market owned the second farm. The tomato varieties grown were HA 3019, Vyta and HA 3108 (an indeterminate variety that required staking). An early planting in September was carried out to obtain a high price at a time when tomatoes were scarce. Tomato fields were rotated to reduce the carryover of pests and diseases, but newly planted fields were close to residues of previous tomato crops. The farmer understood the potential risk of tomato crop residues as an inoculum source, but he was not concerned because he planted TYLCV-resistant varieties. He did not believe that barriers, such as strips of maize, were beneficial under his farming system.

On 20 March a grower who owned a farm near the IIHLD station was visited. TYLCV symptoms were seen in one field. Virus disease symptoms were also seen in late growth on tomato crop residues after harvest

A visit was made on 21 March to several farms in Guines, in the southern part of Habana Province, one of the areas in which the epidemiological survey was conducted. Staff accompanied us from the Ministry of Agriculture- Havana, including the chief extension officer Mr Agustin Hernandez. The farms were located in a coastal area where a tomato-rice rotation is practised and which is separated from a larger vegetable production zone further inland by a belt of sugar cane. The typical farm size is 2 to 2.5 ha, although some farmers have *c.* 10 ha. Fields are large and are cultivated by machine. The selection of fields for the survey was based on smaller blocks within these large fields. Following the summer rice crop, tomato is planted on about 75% of the total area, with the remainder mainly planted to garlic, onion and pepper. In most fields, planting of tomato is not possible before late

November and continues until January. Soils do not generally drain sufficiently until late November and there is a high risk of hurricanes before this month. Approximately 60% of the crop is for processing, with 15% destined for the fresh market. Seed production accounts for the rest (25%).
The susceptible variety Amalia is popular in this area, partly because it has some tolerance to wet conditions. TYLCV incidence is generally low and when infection occurs it is usually late in the season. However, in 1993-94 there was a serious problem with TYLCV. Mr Agustin Hernandez considered that this outbreak was caused by the excessive use of insecticides on both tomato and non-tomato crops and by the abundance of whiteflies on weed hosts. Following this epidemic a campaign was launched to try to control the disease. Training activities were conducted and a series of management recommendations drawn up (including good sanitation and a reduction in spraying of synthetic insecticides). The highly susceptible variety Campbell 28 was withdrawn. He view was also expressed that hurricanes reduced whitefly populations and therefore virus infection. During discussions with Mr Agustin Hernandez it became apparent that the Plant Protection Service has data on TYLCV incidence covering the past ten years or so. In addition, Dr Yamila Martinez and her colleague Madeleine have incidence data from other areas.
Action point: Jany to find out whether it would be possible to use these data, and climatic data from official weather stations, to examine possible patterns of TYLCV incidence (e.g. association with below average number of hurricanes).

INSECTICIDE TRIAL

Recent research both in Guadeloupe and Cuba has raised questions about the efficacy of imidacloprid in preventing transmission of TYLCV and PMV. A trial was designed to assess the effectiveness of treatments of Gaucho and Confidor in reducing disease levels. The trial will be conducted on the experimental farm at IIHLD starting in late 2003/2004). The insecticide treatments are: Gaucho, Confidor, Gaucho + Confidor, and insecticide-free control. A susceptible variety will be used. Due to difficulties in rearing whitefly colonies, whiteflies used for virus transmission will be collected from the field. This requirement places a limitation on the number of plants that can be inoculated. We agreed that twenty plants per treatment would be used.

Survey

The epidemiological survey in the Central and Eastern districts has been largely completed (no. of fields covered?) and *c.* 40% of the fields in the Western district have been surveyed. During our discussions and the field visits it became clear that minor adjustments to the survey protocol were needed in order to take into account differences in tomato production systems between Guadeloupe and Cuba. In view of the large areas of intensive tomato production that are found in Cuba, it was decided to record 'area of contiguous tomato' rather than 'field size'. Similarly, it was agreed that the variable 'distance to edge' would be measured as the distance to the nearest edge of the contiguous tomato area (in the direction of the prevailing wind). These variables could be estimated retrospectively for the fields already surveyed, as assessors had drawn maps on the data sheets.

There was some discussion about the scoring of the 'Barrier variable' and it was concluded that barriers would be scored according to whether a tall crop or other physical barrier occurred at the up wind side of the field (or part field) being surveyed. Thus, a score of 1, 2 or 3 will be given according to the perceived quality of the barrier as it might affect whitefly movement.

Sheltered cultivation will be distinguished as a separate variable, scoring 1 for open field, 2 for so called 'umbrella' shelters and 3 for fully enclosed shelters

An extra column will be added to the datasheet to record whether the variety was tolerant or susceptible to begomoviruses.

The calculation of mean whitefly scores will be modified. Originally, the scores were weighted (to reflect the approximate number values that the scores represent), then averaged, and then converted back to a score. It became clear that the conversion back to a score was causing difficulties because negative values could occur in some cases. As a consequence it was decided not to covert the average back to score.

We considered that crops grown in the summer that were whitefly hosts may have an important influence on incidence of TYLCV in the winter crop of tomato. Therefore, it was agreed that 'summer crops' would be recorded as an additional variable.
For the 'Virus sources' variable, it was decided to assess the presence of weeds that are known virus sources as well as other tomato crops. The weeds, which act as virus sources differ between Guadeloupe and Cuba

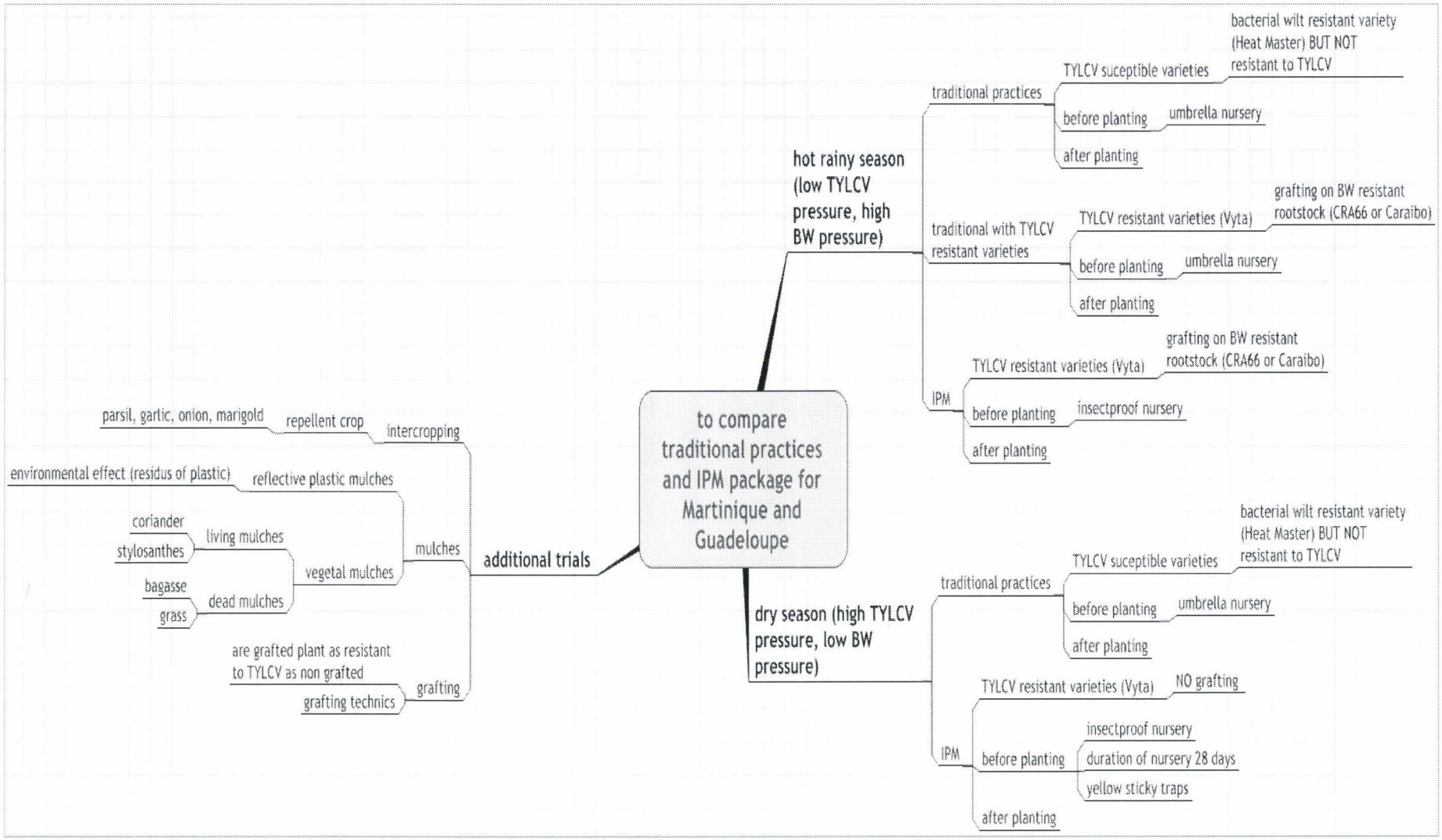
Action points:

(a) *Data to be entered onto the spreadsheet with modifications as described above and in the format provided by John Holt, based on Guadeloupe spreadsheet used by Cecile Marchal,*
(b) *Weed species to be included in the assessment of the virus sources variable to clarified by reference to literature, CENSA/IIHLD*
(c) *Remaining fields to be completed by mid-May. Some of the plots will be in sheltered structures (both open and closed types).*

ITINERARY

Saturday 15 March:	Arrival in Havana at 2145 (CU401)
Sunday 16 March:	Rest day
Monday 17 March:	Visit IIHLD. Discussions with Dr Olimpia Gomez, Antonio Cassanova
Tuesday 18 March:	Field visit to Guira de Melena, Havana
Wednesday 19 March:	Visit CENSA. Meetings with Dr Elsa Martinez de Pol (International Relations), Dr Myra Rodriguez, Dr Yamila Martinez and Dr Olimpia Gomez (IIHLD)
Thursday 20 March:	Visit to IIHLD for further discussions, tour of on-institute tomato production systems using shelters, and field visit to nearby growers
Friday 21 March:	Field visit to Guines, Havana

ANNEXE 4 – IPM STRATEGIES



3 treatments
- traditional practice + suceptible varieties
- traditional practice + resistant varieties (vyta)
- IPM

4 replications
12 elementary plot of 100 plants each
totaly 1200 plants
5 rows of 20 plants each : 6m x 7m = 42m2
total 504 m2, space between plots to be added
between plants on the row 0.3m
between rows 1.4m

before planting, evaluation of % of infected plants on 30% of the plants

2 yellow sticky traps upwind and downwind in each elementary plot (totaly 24 traps) to be changed and counted every week
incidence and severity every week every plot
- incidence : to count number of the 100 plants with symptomes
- severity : to give a score (scale of Scott and Schuster, 92) on 40 plants randomized

flowering : date of 60% plants flowering

fruit set : at the first harvest time. For a cluster, counting number of flowers and of fruits. 10 plants per elementary plot (120 plants), on the 4 first clusters per plant

harvest :
- date of harvest
- commercial according to different size (number and weight)
- rotten : number

economic evaluation
- cost of the plants
- cost of inputs (chemical, biological control, fertiliser...)
- cost of labor
- production value

FINAL DATA SHEET

Contract number: ICA4-CT-2001-10002

Data sheet for final report

(to be compiled by **the co-ordinator** at 12-monthly intervals from start of contract. Figures to be up-dated **cumulatively** throughout project lifetime)

1. Dissemination activities (cumulative)

	Totals	
	Published	submitted
Number of communications in conferences (published)	5	
Number of communications in other media (internet, video,)	9	
Number of publications in refereed journals (published)	21	5
Number of articles/books (published)	1	
Number of other publications	6	

2. Training

Number of PhDs	3
Number of MScs	20
Number of visiting scientists	18
Number of exchanges of scientists (stays longer than 3 months	0

3. Achieved results

Number of patent applications	0
Number of patents granted	0
Number of companies created	0
Number of new prototypes/products developed	8
Number of new tests/methods developed	18
Number of new norms/standards developed	1
Number of new softwares/codes developed	0
Number of production processes	0

4. Industrial aspects

Industrial contacts	no
Financial contribution by industry	no
Industrial partners: - Large	no
- SME 1	no

S. Comments

Other achievements (use separate page if necessary)

' Less than 500 employees.