CHAPTER 3.10

Cuba

Francisco Morales, Gloria González**, Carlos Murguido**, Ana Echemendía**, Yolanda Martínez***, Yenín Hernández***, Benito Faure*[ψ] and *María Chailloux*[ψ]

Introduction

Geographical context

About 25% of Cuba's territory is mountainous, with three distinct mountain ranges running east-west: the eastern (Sierra Maestra), central (*alturas*) and western (Cordillera de Guaniguanico) ranges. The remaining land is composed of extensive plains and basins. Cuba has a semi-tropical climate with two seasons: dry from November through April and rainy from May through October. The mean annual temperature is 26 °C, with a 23-28 °C range. The average precipitation is 1380 mm. All these conditions favour the dissemination of whiteflies, particularly during the dry season of the year, particularly in the regions marked in Figure 1.

Horticultural products have been traditional commodities in Cuba, particularly those referred to as *viandas* and basic grains. These crops include tomato (*Lycopersicon esculentum* Mill.), squash (*Cucurbita* spp. L.), cucumber (*Cucumis sativus* L. var. *sativus*), cabbage (*Brassica oleraceae* L.), sweetpotato (*Ipomoea batatas* [L.] Lam.), eggplant (*Solanum melongena* L.) and common bean (*Phaseolus vulgaris* L.). Vegetable production has become increasingly important to support a rapidly expanding tourist industry in Cuba.

The emergence of Bemisia tabaci *as a pest and virus vector*

The emergence of *Bemisia tabaci* (Gennadius) as a pest is probably linked to the advent and intensive use of agricultural pesticides soon after World War II. *B. tabaci* was reported as a pest of tobacco (*Nicotiana tabacum* L.) and a vector of plant viruses of common bean in the mid 1970s (Blanco and Bencomo, 1978). But it was not until 1989 that *B. tabaci* became a major production problem of tomato and common bean, as a vector of begomoviruses (Murguido et al., 1997). Currently, this whitefly species attacks tomato, common bean, squash, cucumber, melon (*Cucumis melo* L.), cabbage and eggplant throughout Cuba.

By 1990, the new B biotype of *B. tabaci* already had been introduced into Cuba and surrounding Caribbean islands (Brown, 1994). Between 1991 and 1993, the silver leaf syndrome induced by this new biotype was observed on squash (Murguido et al.,

* Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
** Instituto de Investigaciones de Sanidad Vegetal (INISAV), Cuba.
*** Centro Nacional de Sanidad Agropecuaria (CENSA), Cuba.
ψ Instituto de Investigaciones de Hortícolas "Liliana Dimitroya" (LILIANA), Cuba.

Figure 1. The main agricultural regions affected by whitefly-transmitted begomoviruses, Cuba.

1997). Another major production problem for Cuba and the Caribbean region occurred when and Old World virus, *Tomato yellow leaf curl virus* (TYLCV) was introduced from Israel into the Caribbean region, including Cuba (Polston and Anderson, 1997). This exotic virus has caused crop losses worth millions of dollars in the Caribbean region, and it is now known to occur in southern USA and the Peninsula of Yucatán, Mexico.

Advances in Biological Research

A main objective of the research undertaken in Cuba was to determine the composition of the whitefly population. A Cuban trainee, Ms. Yenín Hernandez of the Centro Nacional de Sanidad Agropecuaria (CENSA) conducted the tests at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia, under the supervision of CIAT technical staff. Ninety-nine samples were collected from tomato plants in the localities of Quivicán and Alquizar, Havana Province. The results of the random amplified polymorphic DNA (RAPD) assay showed that 100% of the whitefly samples tested corresponded to biotype B of *B. tabaci*. Additionally, seven whitefly samples from potato (*Solanum tuberosum* L.) plants grown in Havana Province were also shown to consist of *B. tabaci* biotype B. These findings suggest that biotype B has adapted well and now predominates over biotype A of *B. tabaci* in Havana Province.

Bean golden yellow mosaic virus (BGYMV) has been an important disease of common bean in Cuba since the early 1970s (Blanco and Bencomo, 1978). The epicentre of the problem was the locality of Velasco in the western province of Holguín and it is now widely disseminated in the provinces of Holguín, Las Tunas, Guantánamo and Havana. In recent years, the incidence of BGYMV has increased in the provinces of Ciego de Avila, Holguín, Las Tunas and Camagüey.

When the monoclonal antibodies (MABs) to BGYMV were developed in 1990 (Cancino et al., 1995), the Cuban isolate of BGYMV reacted with a monoclonal antibody (MAB 2G5) produced to a Guatemalan isolate of BGYMV, which recognized all of the Middle American BGYMV isolates. By 1993, the Cuban isolates of BGYMV were not reacting with the specific MAB 2G5. As can be observed in Table 1, none of the BGYMV samples from three different provinces of Cuba reacted with the specific MAB-GA (2G5), including a BGYMV isolate from lima

Table 1. Assay of selected common bean samples with a broad spectrum and specific monoclonal antibodies prepared to *Bean golden yellow mosaic virus* (BGYMV), Guatemala, 1993.

Sample	Plant	Locality	MAB-BS[a]	MAB-GA[b]
1	Common bean	Velasco, Holguín	+	-
2	Lima bean	Velasco, Holguín	+	-
3	Common bean (Bonita 11)	Tomeguín, Matanzas	+	-
4	Common bean (Chévere)	Tomeguín, Matanzas	+	-
5	Common bean (Velasco Largo)	Tomeguín, Matanzas	+	-
6	Common bean (BAT 304)	Pulido, La Habana	+	-
7	Common bean	CIAT[c]	+	+
8	Common bean	CIAT[d]	-	-

a. MAB-BS, a broad spectrum monoclonal antibody used to detect bi-partite begomoviruses.
b. MAB-GA, a monoclonal antibody used to detect the original Middle American isolates of BGYMV-Guatemala.
c. Sample 7, BGYMV-GA, Guatemala; CIAT, Centro Internacional de Agricultura Tropical.
d. Sample 8, healthy common bean.

bean (*Phaseolus lunatus* L.). The homologous control, BGYMV-GA, reacted with MAB 2G5 as expected. However, all of the diseased plant samples that were assayed reacted with the broad spectrum MAB (3F7) to BGYMV, which recognizes bipartite begomoviruses.

These tests were repeated in 1997, including some BGYMV-infected common bean plants from the Dominican Republic, a neighbouring Caribbean island (Table 2). The results show that the Cuban BGYMV isolates had changed their antigenic properties, whereas the Dominican BGYMV isolates had not. In order to investigate whether the failure of the MAB-GA to detect the Cuban BGYMV isolates was due to a major change in the capsid protein or putative replicase virus genes, one of the BGYMV isolates from Quivicán was partially sequenced. Table 3 shows the result of this analysis. These results suggest that the Cuban BGYMV isolate has not changed significantly in relation to the original BGYMV isolates from the region and that the Cuban isolate still can be considered a strain of BGYMV.

Table 2. Assay of selected common bean samples with a broad spectrum and specific monoclonal antibodies prepared to *Bean golden yellow mosaic virus* (BGYMV), Guatemala, 1997.

Sample	Plant	Locality	MAB-BS[a]	MAB-GA[b]
1	Common bean	Quivicán	+	-
2	Common bean	Quivicán	+	-
3	Common bean	Quivicán	+	-
4	Common bean	Dominican Republic	+	+
5	Common bean	Dominican Republic	+	+
6	Common bean	CIAT[c]	+	+
7	Common bean	CIAT[d]	-	-

a MAB-BS, a broad spectrum monoclonal antibody used to detect bi-partite begomoviruses.
b. MAB-GA, a monoclonal antibody used to detect the original Middle American isolates of BGYMV-Guatemala.
c. Sample 6, BGYMV-GA, Guatemala; CIAT, Centro Internacional de Agricultura Tropical.
d. Sample 7, healthy common bean.

Table 3. Comparative homologies (%) between a Cuban *Bean golden yellow mosaic virus* (BGYMV) isolate and other common bean begomoviruses.

ORF[a]	BGYMV			BGMV-BR[b]	BDMV-CO[c]
	Guatemala	Dominican Republic	Puerto Rico		
AC1	91.5	91.5	89.8	71.9	69.9
AV1	91.3	91.6	91.3	81.6	78.1

a. ORF, open reading frame; *AC1*, replicase; and *AV1*, capsid protein.
b. BGMV-BR, *Bean golden mosaic virus*-Brazil.
c. BDMV-CO, *Bean dwarf mosaic virus*-Colombia.

With respect to tomato, one of the most affected crops in Cuba, three random samples were take in the locality of Quivicán, Havana Province, and tested for different pathogens (Table 4). As shown, the broad spectrum monoclonal antibody (MAB-BS) used to detect bi-partite begomoviruses did not detect any bipartite begomovirus in the tomato samples, although the observation of two of the samples in the electron microscope revealed the presence of begomovirus-like particles. This result suggested the need to test for the presence of TYLCV, a monopartite begomovirus reported to attack tomato in Cuba (Polston and Anderson, 1997). This introduced virus is best detected by polymerase chain reaction (PCR), using specific primers, kindly provided by D. P. Maxwell and M. K. Nakhla, of the Department of Plant Pathology, University of Wisconsin. The PCR assay detected the presence of TYLCV in two of the tomato samples from Quivican, Cuba.

In 1998, Dr. Gloria González of the Instituto de Investigaciones de Sanidad Vegetal (INISAV), Havana, Cuba, collected samples from symptomatic potato plants in Havana Province. As reported above, potato in Cuba is colonized also by the B biotype of *B. tabaci*. For this test, the viral nucleic acid extracted from affected potato plants was amplified by PCR using the primers developed by Rojas (1992). The amplified region (*AV1* and *AC1*) was cloned and sequenced. Table 5 shows the results of the comparative nucleotide sequence analyses conducted in reference to other whitefly-transmitted begomoviruses.

As can be observed from this comparative test, the begomovirus isolated from potato in Cuba is an isolate of *Tomato mottle Taino virus* (ToMoTV), a virus described in 1997 attacking tomato in Cuba (Ramos et al., 1997). This virus was also closely related to *Bean dwarf mosaic virus* from Colombia (93.3%), *Tomato yellow*

Table 4. Diagnostic assays practiced with three diseased tomato samples from Quivicán, Havana, Cuba.

Sample	EM[a]	MAB-BS[b]	PTY1[c]	CMV[d]	PCR-TYLCV[e]
1	Isometric	-	-	-	+
2	Isometric	-	-	-	+
3	-	-	-	-	-

a. EM, electron microscopy.
b. MAB-BS, a broad spectrum monoclonal antibody used to detect bi-partite begomoviruses.
c. PTY1, potyviruses.
d. CMV, cucumoviruses.
e. PCR-TYLCV, DNA amplification of *Tomato yellow leaf curl virus*.

mosaic virus (ex-Potato yellow mosaic virus) from Venezuela, *Tomato mottle virus* from Florida, USA, *Sida golden mosaic virus* from Costa Rica and *Abutilon mosaic virus* (South American isolate and type species of the cluster in which the Cuban tomato virus is taxonomically placed).

Table 5. Comparative nucleotide sequence homology (%) between a begomovirus isolated from potato in Cuba and previously sequenced whitefly-transmitted begomoviruses.

Region	Virus[a]		
	ToMoTV	ToYMV	SiGMV
Coat protein (*AV1*)	98.5	84.2	85.1
Replicase (*AC1*)	97.7	83.7	82.4

a. ToMoTV, *Tomato mottle Taino virus*; ToYMV, *Tomato yellow mosaic virus*; and SiGMV, *Sida golden mosaic virus*.

Socio-economic Analysis

Cuba has a unique comparative advantage over the rest of the Latin American countries affected by whiteflies and whitefly-borne viruses, specifically, the limited use of agro-chemicals in this island. Moreover, Cuba has been able to develop and successfully implement biological control methods to combat the whitefly *B. tabaci*. One of the most effective entomopathogens produced has been *Verticillium lecanii*. This fungus is very pathogenic to the immature stages of *B. tabaci*. The production of biological control agents in Cuba constitutes an industrial activity in at least 15 provinces of Cuba, where it is being applied on a regular basis to horticultural crops affected by *B. tabaci*. In 1996, 170 tons of the fungus compound were produced in Cuba to treat 12,565 ha of affected horticultural crops. During the peak of the 1992 whitefly epidemics, 29,896 ha were treated in Cuba with this bio-control agent (Murguido et al., 1997).

Tabaquina, a concoction of tobacco leaf residues from the intensive Cuban tobacco industry, is another industrial subproduct used in Cuba to control whiteflies in a highly effective manner. Obviously, the well-planned cropping systems in Cuba also facilitate the implementation of legal measures regulating the time of planting for certain crops which act as reproductive host for the whitefly *B. tabaci* (Murguido et al., 1997).

The control of BGYMV in beans, on the other hand, is effectively carried out through the use of BGYMV-resistant bean cultivars developed in collaboration with the Programa Cooperativo Regional de Frijol para Centro América, México y el Caribe (PROFRIJOL) network and CIAT-Colombia.

Strengthened Research Capacity

The two main Cuban institutions collaborating in the Tropical Whitefly Integrated Pest Management (TWF-IPM) Project were INISAV and the Instituto de Investigaciones de Hortícolas "Liliana Dimitroya" (LILIANA). During Phase I, a third Cuban institute, CENSA, joined the project. The sub-project's coordinator for Cuba, Dr. Gloria González of INISAV, received funds to attend the VII Latin American and Caribbean Workshop on whiteflies and begomoviruses held on 26-30 October 1998, in Nicaragua. Dr. González also visited CIAT, Colombia, for a short but intensive training period on the characterization of whitefly-transmitted viruses.

The TWF-IPM Project contributed to the organization of an International Workshop on Begomoviruses in the Caribbean Region, held in Quivicán, Cuba on 24-29 November 1997. The

project provided three keynote speakers: the coordinator, Dr. Francisco J. Morales; the past co-ordinator of the TWF-IPM Project, Dr. Pamela K. Anderson; and the main support scientist for the Caribbean region, Dr. Jane Polston of the University of Florida. Dr. Anderson explained the nature and objectives of the TWF-IPM Project; Dr. Polston delivered a talk on whitefly-transmitted viruses in the Caribbean Region and disserted on advanced techniques for the characterization of begomoviruses; and Dr. Morales gave a talk on breeding for disease resistance to whitefly-transmitted viruses.

The Whitefly Project also provided funds for the training at CIAT-Colombia of a junior scientist, Ms. Yenín Hernández, of CENSA, Cuba, on the molecular characterization of *B. tabaci* biotypes. CENSA is one of the leading institutes in Cuba in the area of Entomology.

Current Status of Whitefly/Begomovirus Problems

The whitefly situation in Cuba worsened following the invasion of the new B biotype of *B. tabaci*. However, the relatively low use of insecticides and the production of biological control agents in Cuba should maintain the whitefly problem at a manageable level. The Cuban provinces most affected by *B. tabaci* and the viruses it transmits have been Pinar del Río and Havana in the western end of the island and Holguín and Las Tunas in the eastern half of the country. The whitefly problem also has been severe in certain years in the provinces of Santiago and Guantánamo in the eastern end of Cuba. Other provinces affected have been Matanzas, Ciego de Avila, Granma, Camagüey, Villa Clara, Cienfuegos and Sancti Spiritus.

The provinces of Holguín and Las Tunas are located in one of the regions with less precipitation. Also, these provinces have high average temperatures (23.5 ºC) during the winter season (January), conditions that favour the reproduction of *B. tabaci*. The eastern end of the island concentrates the bulk of the tobacco and vegetable production of Cuba, which probably favours the development of large whitefly populations.

The use of entomopathogens, mainly the fungus *Verticillium lecanii*, constitutes a highly effective control measure against the proliferation of *B. tabaci*. Unfortunately, the production of this micro-organism does not meet the needs of the intensive agriculture practiced in Cuba. In the case of common bean, the use of varieties possessing high levels of resistance to BGYMV remains the main strategy for virus control.

References

Blanco, N.; Bencomo, I. 1978. Afluencia de la mosca blanca (*Bemisia tabaci*) vector del virus del mosaico dorado en plantaciones de frijol. Ciencias de la Agricultura 2:39-45.

Brown, J. K. 1994. Current status of *Bemisia tabaci* as a plant pest and virus vector in agro-ecosystems worldwide. FAO Plant Protect. Bull. 42:3-32.

Cancino, M.; Abouzid, A. M.; Morales, F. J.; Purcifull, D. E.; Polston J. E.; Hiebert, E. 1995. Generation and characterization of three monoclonal antibodies useful in detecting and distinguishing *Bean golden mosaic virus* isolates. Phytopathology 85:484-490.

Murguido, C.; Vázquez, L.; Gómez, O.; Mateo, A. 1997. Informe sobre la problemática mosca blanca-geminivirus. Instituto de Investigaciones de Sanidad Vegetal (INISAV), Boletín Técnico no. 5, La Habana, CU. 13 p.

Polston, J. E.; Anderson, P. K. 1997. The emergence of whitefly-transmitted geminiviruses in tomato in the western hemisphere. Plant Dis. 81: 1358-1369.

Ramos, P. L.; Guerra, O.; Peral, P.; Oramas, P.; Guevara, R. G.; Rivera-Bustamante, R. 1997. *Taino tomato mottle virus*, a new bipartite geminivirus from Cuba. Plant Dis. 81:1095.

Rojas, M. R. 1992. Detection and characterization of whitefly-transmitted geminiviruses by the use of polymerase chain reaction. University of Wisconsin, Madison, USA.