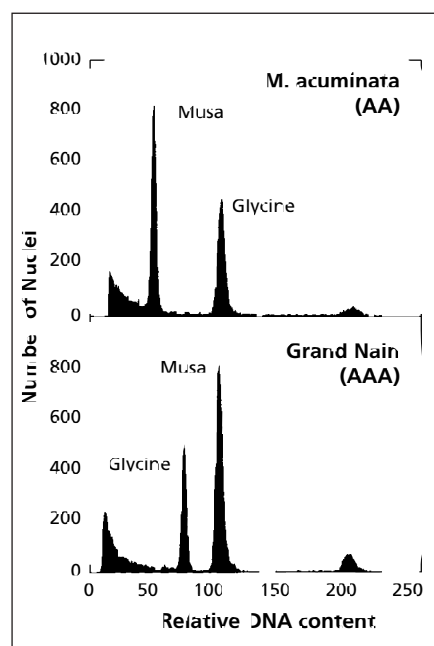


is not destructive – requires small amounts of leaf tissue; 4) it analyses large populations of cells and thus sub-populations differing in ploidy levels can be detected (mixoploidy).

The applications envisaged include: ploidy estimation in new accessions and artificial hybrids, control of ploidy stability after clonal propagation including plants regenerated from *in vitro* cultures, and screening for solid tetraploids after polyploidization. The



**Figure 3.** Examples of flow cytometric ploidy analysis using an internal standard (Glycine max cv. Polanka). For a diploid standard (*M. acuminata* ssp. *banksii*),  $G_1$  peak ratio (Musa/Glycine) was  $196/402 = 0.488$ . The  $G_1$  peak ratio for Grand Nain ( $289/395 = 0.732$ ) was 1.5 times higher thus confirming its triploid status.

first flow cytometers were too expensive and complicated machines to be used only for ploidy screening. Recently, small instruments were introduced to the market with a cost comparable or less than that of a fluorescence microscope. To conclude, *Musa* breeders and taxonomists have now an affordable and reliable tool at hand suitable for ploidy screening in large populations of plants.

#### Acknowledgements

We thank Prof. R. Swennen (INIBAP Transit Centre, Katholieke Universiteit Leuven, Belgium) for the supply of plant material and helpful suggestions, and Mrs. J. Weiserová for technical assistance. One of us, J.D., is grateful to Prof. W. Göhde for providing the Partec PAS II flow cytometer. This work was supported in part by a Research Contract No. 8145/RB from the International Atomic Energy Agency, Vienna. ■

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## Latin America

# The presence of banana streak virus has been confirmed in plantain (*Musa* AAB Simmonds), sugar cane (*Saccharum officinarum*) and edible canna (*Canna edulis*) in Colombia

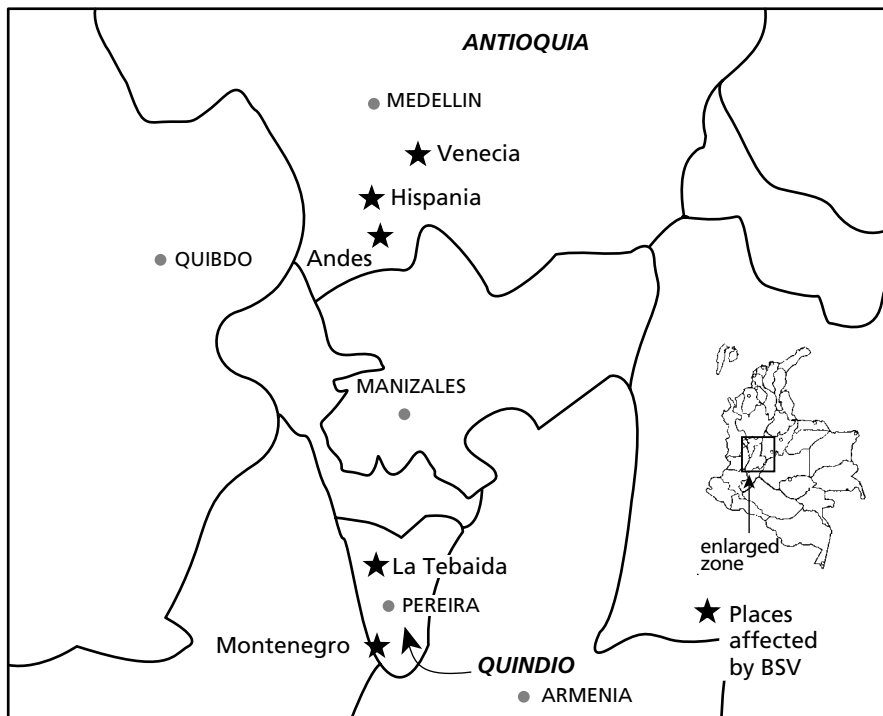
Helena Reichel, Sylvio Belalcázar,  
Gladys Múnera, Rosalía Pérez and  
Emilio Arévalo

Colombia is the world's fourth-largest banana exporter (*Musa* AAA Simmonds) and the seventh-largest sugar exporter (*Sac-*

*harum officinarum*). These products are of great economic importance both for export and local consumption. Seventy percent of production is from the central region where coffee is also grown (Belalcázar 1991, 1992). Banana and sugar cane plantations are often side by side. The two crops are also combined in other countries

(Daniells *et al.* 1995). The only virus disease of banana observed in Colombia is banana mosaic (Belalcázar 1991; Castaño *et al.* 1995a, 1995b), caused by cucumber mosaic virus (CMV). The virus disease affecting the sugar economy is cane mosaic, caused by a potyvirus (Richard *et al.* 1989).

Banana streak disease was de-



Areas in Colombia infected by BSV.

scribed for the first time in Africa in 1974, where it caused damage of up to 90 percent in plantations of 'Poyo' (AAA) Cavendish banana and the pathogen was isolated in 1986 (Lockhart 1986). The symptoms of the disease are chlorotic or necrotic streaks. The plants affected by the disease produce small, deformed bunches and the plant is destroyed in the most serious cases (Jones and Lockhart 1993).

The aim of the work described here was to determine the pathogenic agent of this disease whose aetiology is not well known in Colombia and that is observed in plantations of 'Dominico-Hartón' plantains (*Musa* AAB Simmonds).

#### Material and methods

##### Isolation of the pathogen.

Observation of plantations in the municipalities of Hispania, Andes and Venecia (Antioquia) and in Tebaida and Montenegro (Quindío) revealed leaf symptoms of viral origin in the clone 'Dominico-Hartón'; they consisted mainly of chlorosis that subsequently became necrotic. The municipalities mentioned are at elevations of 1,350 to 1,650 m, with an average temperature of 21 to 22°C. In Andes, samples of sugar leaves with severe mosaic symptoms were collected in a plantation adjoining a field of plantain displaying chlorotic streak systems. In addition, at the same place and also in

Montenegro, leaves of edible canna near plantain fields with chlorotic streak symptoms displayed slight mosaic symptoms.

##### Serology

Plantain, sugar cane and edible canna tissue with apparently viral symptoms were subjected to DAS ELISA immunoenzymatic study (Clark and Adams 1977) using polyclonal antibodies against isolates of BSV-Trinidad Mysore, BSV-Rwanda and BSV-Moroccan (Agdia, Elkhart, Inc. USA).

##### Electron microscopy

Leaf extracts of BSV-infected plantain and sugar cane plants were used for specific serological study using electron microscopy (ISEM) (Roberts *et al.* 1984). The leaf tissues were homogenised to 1:1 (w/v) using a cold phosphate buffer solution 0.01 M, pH 7.6. The mixture was centrifuged at 8,000 g for 15 min at 4°C and the supernatant was collected. A drop of the latter incubated at ambient temperature for 5 hours was placed on a collodion-coated copper electron microscopy grid with a drop of BSV polyclonal antiserum (buffer solution phosphate 0.01 M, pH 7.6) at a dilution of 1:100. The grid was placed on a drop of leaf extract and incubated for 24 hours at 4°C and washed in distilled water. The virus particles were then negative-stained with uranyl acetate 2% every 2 minutes. The virus particles

were observed under scanning electron microscopy (HITACHI HA-12A) with x 50,000 amplification.

##### Purification of the pathogen

A slightly modified version of Lockhart's method (1986) was used. Approximately 50g of infected leaf tissue was sprayed in a mortar with liquid nitrogen. The whole was then homogenised in 170 ml Tris-citrate 0.05M buffer solution at pH 7.4 containing 0.5% (w/v) sodium sulphate, 1% (w/v) polyvinylpyrrolidone (PVP, molecular weight 40,000) and 1% Triton X-100. The mixture was filtered on gauze and homogenised again with chloroform 25% (v/v) for 20 seconds. After centrifugation at 4°C for 10 min at 10,000 g, the supernatant obtained was centrifuged at 136,000 g for 60 min at 4°C. The pellet was resuspended in a cold phosphate buffer solution 0.01 M, pH 7.2 and placed on a continuous sucrose gradient (20-70%) prepared in buffer solution of 0.01 M Tris-HCl and 1 M NaCl. After centrifugation (SW 36) at 30,000 rpm for 3 hours 30 min at 4°C, the band formed was collected with a syringe.

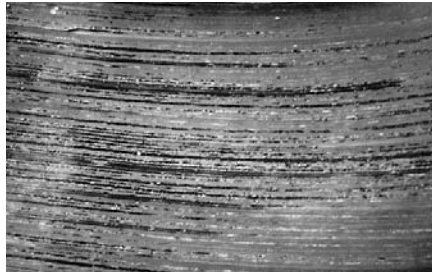
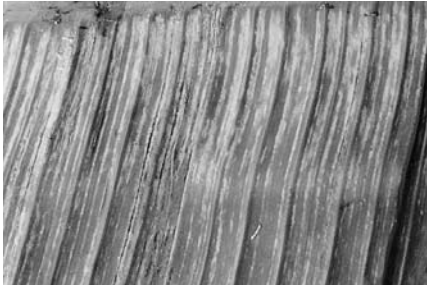
##### Spectrophotometry

A  $A_{260/280}$  absorbance of purification of the pathogen was defined using a Milton Roy Spectronic 601 spectrophotometer.

#### Results and discussion

The symptoms observed in leaves of 'Dominico-Hartón' plantain are similar to those caused by banana streak disease (Jones 1994, Jones and Lockhart 1993, Richard *et al.* 1989). The symptoms detected (Fig. 1A and 1B) in plantain leaves were characterised by chlorotic and necrotic streaking. Occasional swelling and splitting of the pseudostem were observed (Fig. 2) and rosetting (Fig. 3) that subsequently disappeared as in the case of streaking of the pseudostem and the petioles (Fig. 4). The DAS ELISA immunoenzymatic study performed on infected leaf tissue of plantain, sugar cane (Fig. 5A) and edible canna (Fig. 5B) confirmed the presence of BSV in this material. DAS ELISA serological analysis of the various organs of plantain affected by BSV showed that the virus can attack leaves, pseudostem and petioles. Direct electron microscopy examination of plantain and sugar cane leaf extracts revealed the presence of bacilliform viral particles approximately 110 nm long and 30 nm wide and approximately 150 nm long and 30 nm wide respectively (Fig. 6A and 6B).

Purification of leaf extract using a



**Figure 1.** Leaf of 'Dominico Hartón' plantain with chlorotic streaking (A) and necrotic streaking (B) symptoms (photo: A. Ríos)



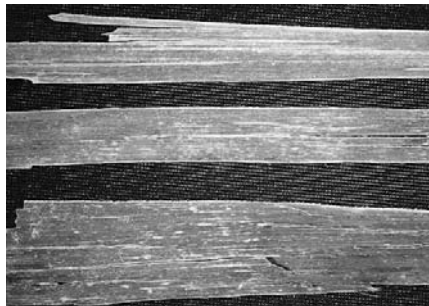
**Figure 4.** Mosaic symptoms on the pseudostem of 'Dominico Hartón' plantain infected with BSV (photo: H. Reichel)



**Figure 6.** Bacilliform virus particles (A) of a 'Dominico Hartón' plantain infected with BSV and (B) sugar cane also infected with BSV. Scale: 30 nm (with the kind permission of R. Pérez).



**Figure 2.** Pseudostem of 'Dominico Hartón' plantain with splitting symptoms (photo: H. Reichel)



**Figure 5.** (A) Leaves of sugar cane (*S. officinarum*) with mosaic symptoms and (B) edible canna leaf (*C. edulis*) with slight mosaic symptoms in the basal part (photo: H. Reichel)

cane in Antioquia and edible canna in Antioquia and Quindío. This is not only the first report of the presence of BSV in plantain (*Musa AAB Simmonds*), sugar cane (*S. officinarum*) and edible canna (*C. edulis*) in Colombia, but also the first report that the latter plant is a host of BSV.

Work is in progress on the characterisation of the virus, as is field work in Andes municipality (Antioquia) to determine the effects of BSV on the growth and production of 'Dominico-Hartón' plantain.

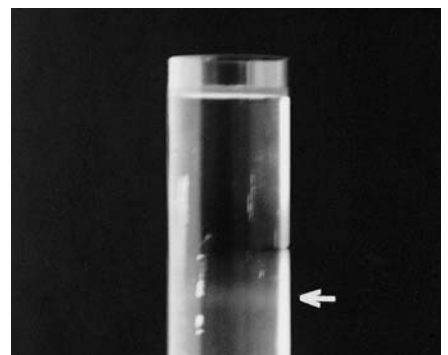
This badnavirus can be controlled by the creation of new plantations using material certified to be free of BSV and preferably located in production regions that are isolated from BSV host plants. It is essential not to enhance the vegetative propagation of BSV-infected material and to remember that this virus is not eliminated by the tissue culture method. BSV is not spread mechanically but in a semi-persistent form by the insect vector *Planococcus citri*. Control of the disease also runs up against the problem of the periodicity of the expression of



**Figure 3.** Rosetting of leaves of 'Dominico-Hartón' infected by BSV (photo: H. Reichel)

continuous sucrose gradient revealed a band formed by virus particles (Fig. 7). These particles gave a spectrum typical of a nucleoprotein (Lockhart 1986), with  $A_{260/280}$  absorbance of 1.26.

The results of the spectrophotometry and electron microscopy serological tests showed that the BSV badnavirus was present in the plants sampled, which also displayed the symptoms described for the virus. Likewise, it was found that BSV infests sugar



**Figure 7.** Purification of the causal agent demonstrates the presence of a band of particles of viral appearance (photo: J. del Carmen Barrera)

the symptoms (Jones 1994). For this reason, it is recommended that plants should be kept in quarantine for 9 to 12 months. Diagnosis of BSV is also difficult because a number of strains are heterogeneous from the serological and genetic point of view.

It is important to continue research on the distribution of this new disease, to identify the BSV races in Colombia and also to determine the pathogenicity of the different isolates or races, the *Musa* germplasm that possesses resistance or tolerance to the disease, the effect of the virus on plantain and sugar cane production and finally the other host plant species and the vectors involved in the spread of the disease.

#### Acknowledgements

The authors thank Dr José del Carmen Barrera, Cristina Suárez, Diana Carrasquilla and Liliana Pachán for their assistance in certain aspects of the study. ■

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### Latin America

## Strategies for the improvement of banana growing in Cuba

S. Rodríguez Morales, J.M. Alvarez, J. de la C. Ventura Martín, J. López Torres, M. García García and J.A. Pino Algora

Bananas and plantains are grown in approximately 120 countries, with total production of approximately  $75 \times 10^6$  tonnes of fruits, most of which are grown by 56 of these countries. Nine-tenths of production is an important food resource for more than 400 million inhabitants of tropical countries and the rest is exported to industrialised countries. Approximately a third of the fruits are plantains and the rest are dessert bananas. Some 50 percent of the world plantain crop is grown in Africa, 25 percent in South America, 15 percent in Asia and 10 percent in Central America (Swennen 1995). Bananas are rich in carbohydrate, vitamins C, B and A and minerals such as potassium and calcium.

In the past two decades, banana and plantain production has been seriously threatened on the one hand by pathogens and on the other by population growth preventing long fallow periods and thus causing decreased soil fertility (Rodríguez 1994).

Black Sigatoka (*Mycosphaerella fijiensis* Morelet) is generally considered to be the major cause of falling yields of both plantain and dessert banana (Ortíz *et al.* 1995, Mobambo *et al.* 1993, Craemer & Ortíz 1995). The variability of the reaction observed in tetraploid progeny of plantain suggests that black Sigatoka could be controlled by the use of recessive genes.

The banana borer *Cosmopolites sordidus* Germar is of Indo-Malaysian origin and widespread in the tropics. The

pest affects both plantain and banana crops by weakening the corms and causing toppling. The borer, combined with nematodes that also cause serious damage to roots, reduces nutrient absorption and transport, causing decreased plant growth that in particular affects fruit filling.

The causes of the decrease in yields are thus complex. In addition to those listed above, it should be mentioned that banana plants may topple fairly easily under certain field conditions, for example when growth of the root system is hindered or when bunch weight increases. Although the latter feature enhances productivity, it also increases the risk of stem breakage, not to speak of the tendency for the corm to develop closer to the surface of the ground in certain economically important clones.

When the positive and negative points of banana and plantain growing are examined, it seems essential for Cuba to set up a genetic improvement programme which, combined with ecologically sustainable and appropriate technology would make it possible to seek solutions to these problems in the various existing cropping systems.

#### Plantain and banana in Cuba

Plantains and dessert bananas traditionally form a large part of the Cuban diet and were an important feature in exports to the United States in the last decades of the nineteenth century (Balmaseda 1886). This commercial activity began to decrease with the appearance of banana wilt (*Fusarium oxysporum* f. sp. *cubense*) and then exports stopped in 1950 when the foreign companies abandoned the banana plantations. The companies transferred their capital to Central America where