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CIRAD INVASIVE SPECIES INITIATIVES IN THE CARIBBEAN BASIN

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ABSTRACT. Several research programmes of CIRAD (Centre de Coopération en Recherche Agronomique pour le Développement) target invasive species in the Caribbean. These programmes deal primarily with pathogens (bacteria, bacteria-like, fungi and viruses) of major crops grown in the French West Indies (Guadeloupe and Martinique) and elsewhere in the Caribbean, such as banana, sugarcane and coconut. CIRAD has developed new tools for the detection of some of these pathogens: Ralstonia solanacearum (causal agent of Moko disease), Mycosphaerella fijiensis (causal agent of Black Sigatoka disease, BSD), Banana streak viruses (BSV), Banana mild mosaic virus (BanMMV) and banana virus X (BVX). Using these and existing detection tools, the presence, prevalence and diversity of pathogens established on or threatening banana, sugarcane and coconut in the Caribbean were investigated. These investigations resulted in recommendations for the control of the above-mentioned pathogens. Also, several of the detection techniques developed in these investigations were successfully transferred to Caribbean Plant Protection Services, paving the way for the development and implementation of a regional surveillance network of crop diseases.

KEY WORDS: crops; invasive species; Ralstonia solanacearum; Black Sigatoka; coconut lethal yellowing; Sugarcane yellow leaf curl virus; Banana streak viruses; diagnostic; surveillance networks.

INTRODUCTION

CIRAD has developed several initiatives on invasive plant pests and pathogens that are present in the Caribbean. These initiatives are primarily focused on Ralstonia solanacearum, Black Sigatoka, coconut lethal yellowing and viral diseases of sugarcane and banana. They include (i) research activities, (ii) transfer of diagnosis techniques to plant protection and quarantine services and (iii) participation in surveillance networks, either existing or under construction.

Epidemiological and molecular studies are the key component of many CIRAD research activities in the Caribbean. They are often carried out in the frame of international collaborative projects, such as surveys undertaken in Grenada and St Vincent on banana Moko disease. Epidemiological studies were also carried out in Guadeloupe and Martinique on several pathogens affecting sugarcane, such as sugarcane yellow leaf virus and leaf scald disease. These studies have helped implement strategies for controlling established and emerging diseases and pests of sugarcane. Likewise, epidemiological and diversity studies carried out on coconut lethal yellowing, Black Sigatoka and Banana streak viruses helped in the establishment of control strategies.

Diagnosis and monitoring tools and techniques are one of the major outputs of CIRAD research activities. Transfers of these tools and techniques to plant protection and quarantine services of Caribbean countries are a key component of the CIRAD's strategy, which aims at increasing food security in the Region through better control of pathogens. These transfers are achieved through collaborative projects and regional workshops. Such workshops were successfully organised in Guadeloupe in order to transfer techniques for the detection and monitoring - for example - of Black Sigatoka and the detection of several viruses infecting *Musa* species.

CIRAD also plays an active role in global surveillance networks such as PANDOeR and the current USDA/CARICOM joint initiative for promoting plant health in the region through existing networks (CISWG, CISSIP). CIRAD's current projects involve the development of a Regional Black Sigatoka surveillance and control network, and a participatory database on major diseases of banana, coconut, horticultural crops, sugarane and yam.

Moko disease

Moko disease is an ancient disease in the Caribbean. Moko disease was first described in Guyana in 1840, then reported during devastating outbreaks in 1890 in Trinidad (Rorer, 1911), and it reached the industrial banana plantations of Central America in the 1950s (Sequeira, 1998). It is now emerging and spreading over the Caribbean, affecting Jamaica since 2003, and St Vincent since 2007.

Moko disease is caused by specific strains of the soilborne beta-proteobacterium, *Ralstonia solanacearum* (formerly called race 2). The most recent phylogenetic analyses demonstrated that this bacterial species should be considered a species complex composed of four phylotypes related to geographical origin : I = Asian, II= American, III=African, IV=Indonesian (Fegan & Prior, 2005, Prior & Fegan, 2005). The robustness and reliability of this 4 phylotypes scheme was clearly demonstrated by recent genomic studies using a DNA chip microarray approach (Guidot *et al.*, 2007). Moko-inducing strains are distributed within phylotype II in four genetic groups named sequevars: sequevar 3 originating from Central America, sequevar 4 from Peru and Colombia, sequevar 6 from Venezuela and sequevar 24 being specific to Brazil (Fegan & Prior, 2006).

Thanks to the recent development of strain-specific identification tools, field surveys were carried out in Martinique and Grenada. They showed that Moko-related strains can also threaten vegetable and flower crops. In Martinique, phylotype II/sequevar4NPB strains, which are not pathogenic on banana but do belong to the Moko strain's genetic group, are pathogenic on anthurium, Heliconia and cucurbits, and are spreading rapidly on tomato (Wicker et al., 2007, Wicker et al., 2009).

In Grenada, where Moko disease has been described since 1978 (Ambrose, 1987), Moko strains were found to induce insect-transmitted and soil-borne infestations; all were assigned to sequevar 6 following multiplex-PCR gene sequences analyses; whereas, R.solanacearum strains pathogenic on vegetables (particularly tomato) were all assigned to sequevar 5. Molecular diagnosis studies thus showed that Moko disease was re-emerging on the island, due to the loss of know-how on disease prevention among farmers. This loss of understanding and skills is the result of failure of older experienced growers to transmit to young growers the basic preventive measures they had learned during the early 1980's.

Mapping of the different Moko groups within the Caribbean subregion is essential to the implementation of effective control strategies, since it provides important data on the origin of strains, leading to the identification and quantification of pathogen flows. To this aim, such a mapping is currently being undertaken in the West Indies, in the frame of collaboration between the University of the West Indies (St Augustine) and CIRAD Réunion.

Black Sigatoka disease

Mycosphaerella leaf spot diseases are the most damaging and costly diseases of bananas and plantains (Jones, 2000) especially for banana export production. They are due to ascomycete fungi whose ascospores are naturally spread by wind. Mycosphaerella fijiensis, is responsible for the Black Sigatoka (BS), and Mycosphaerella musicola is responsible for Sigatoka Disease (SD). These foliar diseases affect yields and fruit quality. BS is more severe than SD because it affects plantains and its infectious cycle is shorter (Jones, 2000). Control against Mycosphaerella leaf spot diseases rely on frequent aerial fungicide sprays. On average, 10 annual fungicide sprays are necessary to control SD in the French West Indies, whereas 30 to 60 sprays are necessary to control BS, depending on environmental conditions and spraying strategy.

All major banana producing countries in Latin America, the Caribbean and Africa are affected by BS, except several Caribbean islands (French and English West Indies) which are affected by SD but remain free of BS so far. BS as first described in Fiji in 1963 and has been spreading worldwide rapidly since, probably by means of transportation of infected bananas plants. It was first detected in Latin-America (Honduras) in 1972 and in the Caribbean (Cuba) in 1990 (Jones, 2000). Then it spread to Jamaica (1995), the Dominican Republic (1996) and Haiti (1999). More recently, it was described in 2003 in Trinidad (Fortune et al., 2005), in 2004 in Grand Bahama island (Ploetz, 2004) and Puerto-Rico (Irish et al., 2006). The last emergence in the Caribbean was in 2005 in Grenada (Graham, 2007).

Because of the above-mentioned recent history, BS is considered to be an emerging disease. It currently threatens banana production in several Caribbean areas and its introduction in disease-free areas such as the French West Indies and Windward islands could have a devastating effect on their banana production. Therefore, CIRAD's initatives on BS aim at a better control of the disease through 3 kinds of activities as follows:

- **BS surveys.** Surveys consist in analysing banana areas, collecting infected leaf samples and performing visual and molecular diagnosis. Four surveys were realised in 2004 in Saint-Lucia, Saint Vincent, Dominica and Suriname, following the request of banana producers' organizations. Twelve to twenty samples were harvested in each country and analysed by morphotaxonomic observations and molecular diagnosis. No positive samples could be identified at that time.
- **Assistance and training in BS diagnosis.** Two kinds of diagnosis were developed by CIRAD. The first one is based on morphotaxonomic observations, i.e observation of conidia and conidiophores in order to differentiate M. fijiensis from M. musicola (Zapater et al., 2008). It requires the occurrence of sporulating lesions. A molecular diagnosis was also developed, based on a method developed in Australia (Johanson et Jeger, 1993). It allows an early detection of the disease and is therefore essential to surveillance. CIRAD has taken part in symptom recognition workshops organised by Wibdeco (banana organization from the Windwards) in 2004 in Saint-Lucia, Saint-Vincent and Dominica. About 100 persons from Ministries, producer organisations and quarantine services were trained. CIRAD organised in 2008 two BS diagnosis trainings for plant protection staff: one was organised at CIRAD Guadeloupe, with participants from Saint-Vincent, Dominica and Saint-Lucia; another took place at CIRAD's headquarters in Montpellier (France), with participants from the 4 French overseas departments (Guadeloupe, Martinique, French Guyana and Réunion Island). Thus, several key persons from the 5 currently BS-free banana countries have skills to detect BS at early stages.
- **Initiative to set up a BS surveillance network in the Caribbean**
    - FWI network: based on its experience on BS, CIRAD has taken part in the implementation of a BS surveillance network in the FWI (see the paper of J. Iotti et al.). This network aims at surveying all banana areas (commercial and backyards) in order to detect potential BS introduction as early as possible and eradicate it. It started operating in April 2008 in Martinique and will startoperating in Guadeloupe in 2009. It is coordinated by the French Plant Protection services and involves CIRAD, FREDON and banana producers' organisations.
    - Regional network: in October 2006, CIRAD organised a BS regional workshop in Guadeloupe, with 32 participants from agriculture services and banana producers from 13 Caribbean countries. The main output of this workshop was the implementation of a Caribbean BS network, which aims at increasing the exchange of information and at promoting collaboration between Caribbean countries. There is no official structure yet but CIRAD plays a key role in this regional network. Some specific needs were identified, such as disease surveillance, diagnosis and development of alternative control methods such as resistant varieties and use of biofungicides.

Finally, a research project coordinated by CIRAD, funded by the French National Research Agency (ANR) and called ANR Emerfundis was started in 2008. It aims at unraveling the dynamic of M. fijiensis populations in the Caribbean, based on the analysis of historical data and genetic structure of fungal populations. All Caribbean banana countries where BS has been reported are taking part in this project by collecting historical data and, in some cases, collecting biological samples. Historical data are currently being processed whereas molecular analyses are planned for 2010.

LETHAL YELLOWING DISEASES

Lethal yellowing diseases are caused by phytoplasmas. They have destroyed coconuts by the thousands in the Caribbean region and CIRAD has put a priority on research on these diseases (Dollet 2002). CIRAD's interventions focus on three factors: (i) the pathogen and the etiology of the disease itself, (ii) the vector and finally, (iii) search for sources of genetic resistance in coconut.

In the family of Lethal yellowing diseases, several types can be distinguished depending on symptoms and epidemiology (Dollet et al. 2008). They are caused by various phytoplasma strains that can be identified using heteroduplex mobility assay (Marinho et al. 2008). CIRAD collaborated in research conducted to characterize the pathogen and the development of the disease in Cuba (Llauger et al. 2006), the Dominican Republic (Martinez et al. 2008) and Jamaica (Myrie et al. 2003; Myrie et al. 2007).

Although Myndus crudus has been identified as a vector of the disease, search for possible alternative vectors are still needed and CIRAD collaborates with CICY in Mexico in this domain (Julia et al. 2008). CIRAD also contributes to a better knowledge of the ecology and diversity of Myndus crudus and related species, in collaboration with CIB in Jamaica (Brown et al. 2002, Brown et al. 2008).

At the turn of the century, an outbreak of the disease occurred in Jamaica. It affected varieties that were, so far, considered resistant (at least partially) to the disease. One possible cause for this outbreak was that the planting material that was used was not true to the varieties that were evaluated for resistance in the 70's. Molecular characterization showed that, although some deviations existed, they were insufficient to explain the intensity of the recent attack (Baudouin et al. 2008; Lebrun et al. 2008). This indicates that changes occurred also in pathogens or vectors.

BACTERIAL AND VIRAL DISEASES OF SUGARCANE

CIRAD has developed epidemiological research programmes on several diseases that are affecting sugarcane in the Caribbean. Most of these studies are focused on the variability of pathogen populations and their dispersion patterns, including studies on climate conditions favouring this dispersion. These programmes are primarily focused on two pathogens;

- Xanthomonas albilineans, the causal agent of sugarcane leaf scald, is mainly transmitted by harvesting tools and stalk cuttings. However, this bacterial pathogen can also be transmitted by aerial means, and aerial spread is an important step in the disease cycle. Recent studies showed that the amount of rainfall during the wet season is a key factor in leaf scald epidemics and disease progress in Guadeloupe (Champoiseau 2009).
- Sugarcane yellow leaf virus (SCYLV), the causal agent of an emerging aphid vectored disease called yellow leaf, is present in numerous sugarcane countries

worldwide. In the Caribbean, the virus was first identified in the French West Indies in 1996. SCYLV was diagnosed later at CIRAD's sugarcane quarantine facility in Montpellier in seed cane originating from Barbados and Cuba (Daugrois, 2008). It was also detected in Belize and Jamaica lately during a survey realized by Cirad Guadeloupe. Recent studies showed that genetic variation occurs within SCYLV, and four genotypes of the virus differing in aggressivity and virulence have been reported so far (Abu Ahmad et al., 2007). Characterization of SCYLV genotypes and vector population dynamics are essential to analyse the risk of yellow leaf epidemics. To this aim, CIRAD has undertaken a study of yellow leaf incidence in 34 farmers' fields, and aphid vector populations and distribution of SCYLV genotypes in the two closely related islands of Guadeloupe and Martinique. Important differences in disease incidence and frequency of virus genotypes between both locations were unveiled, suggesting the occurrence of strong local effects on virus populations that need to be further characterized. Therefore, disease progress in one location cannot yet be predicted based on the situation in another relatively close location, and the status of yellow leaf disease must be studied locally in order to determine the risk of epidemics and to measure its impact on sugarcane production.

Results from research programmes fuel the development of sugarcane crop protection strategies. For example, the implementation of pathogen detection techniques help CIRAD to promote the safe movement of sugarcane germplasm through varietal exchange performed under the control of the International Sugarcane Quarantine, which is located at CIRAD's headquarters in Montpellier (France) and operated by the French plant protection services. CIRAD has also developed tools for distance diagnosis of ratoon stunting, yellow leaf and leaf scald diseases by means of tissue blot that can be sent by mail for diagnosis, avoiding the need to send plant samples. This technique was used succesfully for disease diagnosis in Jamaica (Falloon, 2006), Belize and the Dominican Republic.

CIRAD also focuses on the genetic characterization of resistances against sugarcane pathogens and on phenotyping its sugarcane germplasm collection for such resistance, in order to develop disease resistant hybrid varieties.

VIRAL DISEASES OF BANANA

Of the 8 characterized virus species affecting banana and plantain, 4 are present in the Caribbean: Banana mild mosaic virus (BanMMV), Banana streak viruses (BSV), Banana virus X (BVX) and Cucumber mosaic virus (CMV). CIRAD has research activities on the three former. These activities are primarily focused on the characterization of the diversity of viral populations, in order to develop and/or optimize detection techniques that are both sensitive and polyvalent, and that can be used to assess the levels of prevalence of virus species in banana and plantain plantations.

Diversity studies were carried out on BanMMV (Teycheney et al., 2005a) in Guadeloupe. They incidentally led to the discovery of a new virus species, Banana virus X (BVX) in Guadeloupe

(Teycheney et al., 2005b). This research resulted in the development of distinct detection techniques adapted to the diversity of both viruses (Teycheney et al., 2007). Likewise, optimized detection techniques were established for BSV (Le Provost et al., 2006), in order to minimize the risk of detecting false positives resulting from the presence of endogenous BSV sequences that are present in the genome of some banana and plantain cultivars (Hohn et al., 2008). Using such detection tools, prevalence studies were carried out for BSV and BVX in Guadeloupe (Perefarres et al., in press) and for BSV in Cuba (Javer et al., in press), leading to recommendations on the control of these viruses.

Transfer of detection techniques to plant protection services in the Caribbean are an essential part of CIRAD's strategy, which aims at increasing food security in the region through a better control of pathogens. It is also essential that these techniques are widely transferred to and used by private companies multiplying planting material, especially vitroplants, in order to avoid outbreaks resulting from the large scale distribution of virus-infected plants. To this aim, several workshops were organized, with staff from plant protection services and private companies attending.

CONCLUSIONS

Invasive species are threatening health, agriculture and food security worldwide, especially in tropical insular environments such as the Caribbean, where they can spread very rapidly and become endemic. Their control requires coordinated efforts from scientists, protection services and private operators.

CIRAD has developed research programmes focused on several diseases and pathogens of major crops in the Caribbean, with research teams based in the French West Indies and in mainland France. These research programmes aim primarily at increasing our knowledge on pathogens diversity and population dynamics. They are leading to better diagnosis tools and surveillance strategies that need to be implemented both at national and regional levels, in order to prevent the spread of existing pathogens and the introduction of new ones. To this aim, CIRAD has developed an extensive collaborative network with research institutions and plant protection services in the Caribbean. CIRAD is also taking part in initiatives aiming at the development of surveillance networks of pathogens in the Caribbean.

Increased movements of the human population and goods, global warming and environmental changes deeply affect the dynamics of diseases and the rate of emergence of new diseases. Public attention has been focused on the recent emergence or re-emergence of animal and human diseases such as AIDS, SARS or Bluetongue. However it is expected that similarly important diseases affecting crops will emerge at an accelerated rate in the future, although it should be kept in mind that they have never stopped emerging (Anderson et al., 2004). Global response is therefore needed in order to face this global threat. One of the keys to success is the implementation of regional coordinated strategies including the development of unique surveillance networks for crop diseases and pests, data exchange and joint response strategies.

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