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Plant Virus and Phytopathology Research in Jamaica: A Review

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ABSTRACT

During the last twelve years, a number of plant viruses have emerged in Jamaica resulting in severe yield losses and potential threats to various economically important crops. Numerous begomoviruses have been characterized from several crops including tomato, red kidney bean, cabbage, papaya and several common weeds. *Papaya ringspot virus* (PRSV) is the most damaging virus pathogen to papaya and is presently a problem due to the intensification of the crop in recent years. Although *Citrus tristeza virus* (CTV) was first detected in Jamaica in the late 1950s, the pathogen currently poses a threat to the Jamaican citrus industry since the severe strain of CTV and one of its most efficient vectors, *Toxoptera citricida*, were recently confirmed in Jamaica. Research aimed at the distribution of these plant viruses affecting major agricultural crops and the structure of the virus populations is ongoing. The subject of this review is an analysis of the genetic diversity in the three virus populations and factors contributing to their emergence. The challenge is to complement this analysis with appropriate diagnosis, quarantine activities and management of the diseases.

Keywords: *Citrus tristeza virus*, Geminivirus, *Papaya ringspot virus*

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INTRODUCTION

In Jamaica, agriculture is the third largest foreign exchange earner, after tourism and mining, and the second largest employer of labour. Like many regions in the Caribbean, the origin of Jamaican agriculture was based on the large scale cultivation of a few crops, such as sugar cane, banana and coffee, for the international market. There was some small-scale cultivation of tropical vegetables for the domestic market.

Although the structural dichotomy still exists today, Jamaica over the past few decades has embarked on a diversification program and has been seeking markets for several non-traditional agricultural products with some success. The small scale sector of the industry which presently accounts for a large proportion of farm labour produces a wide range of crops. Yam, potato, dasheen, coco, plantain, cassava, red kidney bean, and pumpkin are a few of the root crops and tropical vegetables in production. Several of these crops have been targeted for export. The country is currently competitive in the production of citrus and there has been some success in penetrating the international market with papaya.

However, susceptibility to pests and diseases is threatening the sustained production and continued expansion of the agricultural sector of the country. A comprehensive review of the ecology of virus diseases of crops in tropical countries was recently published (Loebenstein and Thottappilly 2003). In addition, the features of the tropical environment that affect the development and progression of plant diseases in this region have been described (Thresh 1991; Fargette *et al*. 2006). This report focuses on the events that have led to the emergence of and research on three economically important viruses in the *Geminiviridae*, *Potyviridae*, and *Closteroviridae* in Jamaica.

Geminiviruses

Geminiviruses (*Geminiviridae*) are insect vectored plant viruses that have impacted the production of many crops including cassava and maize in Africa, cotton in Pakistan and tomato in several regions including the Mediterranean and Latin America (Varma and Malathi 2003; Legg and Fauquet 2004). Various species of geminiviruses have been reported present in Jamaica since the mid 1970s, plaguing both economically important crops and weeds alike.

Geminiviruses contain single stranded DNA genomes between 2.5-3 kb which may be monopartite including the genera *Mastrevirus*, *Curtovirus*, *Topocuvirus* and a few viruses in the genus *Begomovirus* or bipartite which include most of the begomoviruses (Rybicki *et al*. 2000). The genome of bipartite begomoviruses are designated DNA-A and DNA-B and both are required for systemic infection. The DNA-A component of bipartite geminiviruses encodes five genes, one in the virion sense and four in the complementary sense. The virion and complementary sense genes are separated by an intergenic region (IR) of about 200 nucleotides. The IR is unique to each virus but is almost identi-

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cal in both DNA components of begomoviruses and consists of conserved elements important for replication and transcription including the origin of replication. The *Rep* gene encodes the replication initiator protein (Rep) which cleaves a conserved motif at the origin of replication to initiate viral replication (Lazarowitz 1987). The replication enhancer protein, REn, enhances replication possibly by stabilizing the interactions between the viral replication protein and the viral DNA (Fontes *et al*. 1994). The virus coat protein (CP) determines vector specificity. It has no role in replication, transport or symptom development in the bipartite geminiviruses, but is involved in systemic spread and symptom development in some monopartite viruses (Lazarowitz 1987). The transcriptional activator protein, TrAP transactivates the coat protein gene (*CP*) and the nuclear shuttle protein gene (*NSP*) which is encoded on the DNA-B component (Sunter and Bisaro 1992; Bisaro 1996). The exact function of the AC4 protein is not clear but was recently shown to be a suppressor of gene silencing in a few geminiviruses (Vanitharani *et al*. 2005).

The DNA-B encodes two genes, the *NSP* and the movement protein gene (*MP*), which are both involved in movement of the virus. The NSP attaches itself to the newly replicated ssDNA and transports it from the nucleus to the cytoplasm. The MP modifies the size exclusion limit of the plasmodesmata and this allows the virus to infect adjacent cells (Sanderfoot and Lazarowitz 1995). All the genes required for replication, transmission and movement are encoded on the single component of the monopartite geminiviruses (Delatte *et al.* 2005). Recently it was shown that some monopartite geminiviruses of the eastern hemisphere are associated with subgenomic DNA components, sometimes referred to as DNA-β. These components were found to be a requirement for the efficient systemic spread and symptom development (Saunders *et al.* 2000; Briddon *et al.* 2001).

In Jamaica, geminiviruses have been found to infect important crops and many common weeds. In particular, the production of cabbage (*Brassica oleracea*), tomato (*Lycopersicon esculentum*), hot pepper (*Capsicum chinense*) and red kidney bean (*Phaseolus vulgaris*) have been adversely affected by several begomoviruses. Yellow mosaic symptoms of red kidney bean and lima bean (*P. lunatus*) were the first to be reported by Pierre (1974). In the early 1990s the causal agent was identified as *Bean golden yellow mosaic virus* and has contributed to significant reductions in bean production on the island (McLaughlin *et al*. 1994; Roye 1996). Around the same time severe leaf curling and stunted growth was observed on tomato plants in the parishes of St Elizabeth and Manchester in the south central region of the island. Two whitefly transmitted geminiviruses, *Tomato yellow leaf curl virus* (TYLCV) and *Tomato mosaic Havana virus* (TMoHV, previously referred to as *Tomato dwarf leaf curl virus*) were found infecting tomato and Scotch bonnet pepper (McGlashan *et al.* 1994; Roye *et al.* 1999b). The two viruses can occur in mixed infections and in tomato illicit the disease phenotype commonly referred to as the "Jherri curl" disease by local farmers. Of the two tomato viruses TYLCV is the more debilitating and was responsible for the drastic reduction in tomato production in the 1990s (Roye *et al*. 1999b). Begomovirus infection was less frequent in Scotch bonnet pepper and along with *Tobacco etch virus* is a contributory factor to the low productivity of peppers in Jamaica.

Additionally, in 1994 some farmers in Douglas Castle in the central parish of St. Ann complained of severe leaf curling and chlorosis on cabbage plants. Investigations revealed that the symptoms were due to infection by the *Cabbage leaf curl virus* from Florida (CaLCuV-Flo). The cabbage leaf curl disease caused the leaves of the cabbage plant to fold, forming a distorted "cabbage head" which is not saleable (Roye *et al*. 2003). The cabbage virus was subsequently detected in the central parishes of St James, Clarendon and St. Elizabeth infecting cabbage and cauliflower (Smith 2005). A begomovirus was also partially characterized from papaya (*Carica papaya*) in St. Catherine and Clarendon. DNA sequence analysis suggests that the papaya virus is a previously unreported begomovirus and can occur in mixed infections with *Papaya ringspot virus* (PRSV).

In Jamaica, weed species representing at least eleven genera and four families (*Euphorbiaceae*, *Malvaceae*, *Nyctaginaceae* and *Fabaceae*) have been confirmed as geminivirus hosts. Work on the molecular characterization of weed infecting geminiviruses in Jamaica is in its early stages. Partial nucleotide sequences encompassing the 5′ terminals of the *Rep* and *CP* and the IR of the DNA-A and 5′ terminals of the *MP* and *NSP* and the IR of the DNA-B are available for the geminiviruses identified thus far.

The majority of local weeds that are geminivirus hosts belong to the *Malvaceae* family. These include *Kosteletzkya pentasperma*, *Malachra alceifolia*, *Malvastrum americanum*, *Sida acuta*, *Sida spinosa* and *Wissadula amplissima. K. pentasperma* is one host of the Macroptilium golden mosaic virus-Jamaica Strain 1 (MGMV-[JM1]) which also infects *Macroptilium lathyroides* (Roye *et al*. 1999a) and *W. amplissima. M. alceifolia* hosts the Tobacco leaf curl Cuba virus (TbLCuCUV) which infects tobacco in Cuba (Morán *et al.* 2005; Hall *et al.* 2006). *M. americanum* is a host to two distinct geminiviruses, provisionally named Malvastrum yellow mosaic Jamaica virus (MYMJV; Graham *et al*. 2007) and a previously unreported partially characterized virus (A. Graham, pers. comm.). MYMJV was also partially characterized form *Sida spinosa* (Graham *et al.* 2007). *Sida* spp. and *M. lathyroides* host Sida golden mosaic Jamaica virus (Roye *et al.* 1997). Five distinct geminiviruses were found associated with *W. amplissima*; MGMV-[JM1], Wissadula golden mosaic virus (WGMV; Roye *et al*. 1997), Wissadula golden mosaic St. Thomas virus (WGMSTV; Collins *et al.* 2007), SiGMJV and a begomoviruses closely resembling Pepper mosaic Jalisco virus (DQ520942). In the *Euphorbiaceae* family, the weeds *Euphorbia heterophylla* and *Jatropha* sp. are associated with begomoviruses. The geminivirus infecting *E. heterophylla* is a strain of Pepper mosaic Jalisco virus. *J. gossypiifolia* and *J. multifida* are hosts to the tentative species Jatropha mosaic virus (Roye *et al*. 2006). This virus also infects *Jatropha* species in Puerto Rico (AF058025). DNA sequence analysis suggests that *Boerhavia coccinea* (*Nyctaginaceae*) is associated with a previously unreported begomovirus and DNA hybridization and PCR have detected geminiviral DNA associated with *Centrosema* sp.

With the exception of TbLCuCUV-[JM] and the isolate of Pepper mosaic Jalisco virus, all the geminiviruses isolated from local weeds seem to be endemic to the island. Although local weeds are rich sources of begomoviruses, the virus sequences isolated suggest that the weeds discussed herein are not natural alternate hosts or reservoirs of geminiviruses that infect Jamaican crops. Host range studies are needed to show whether the begomoviruses associated with weeds in Jamaica can infect crop plants. Host range determination and biological characterization of the novel begomoviruses infecting weed species could provide insight into the potential of these viruses becoming pathogens of crops in the future. Since three weed species, *M. americanum*, *W. amplissima* and *M. lathyroides*, have been found to host multiple begomoviruses and mixed infections have been detected, there is opportunity for viral recombination and the generation of new geminiviruses (Padidam *et al*. 1999).

Tomato and cabbage producers in Jamaica utilize a number of strategies including cultural practices, chemical control and planting resistant cultivars, for the management of geminiviruses. During the 1990s when the incidence of geminivirus infection seemed to be at its peak, most farmers cultivated the tomato varieties 'Flora Dade', 'Tropic', 'Duke', 'Oxcart', 'Roma' and 'UC82B' and practiced rouging. Given the susceptibility of these varieties to TYLCV, tolerant varieties that are available on the international market have replaced these varieties. They include 'Tropical Glory', 'Summer Star', 'Striker', 'Gemstar', 'Gempear', 'Gempack', 'Gempride' and 'Adonis'. Additionally 'Ala-

sua', large, winter and early types are being evaluated for resistance to TYLCV at the government research institution, Bodles Agricultural Station. There are reports that the incidences of, and yield losses from the "jherri curl" disease have been significantly reduced for those farmers growing these tolerant tomato cultivars. The use of corn as border crop for the protection of tomato and pepper plants is also a common practice used in the management of geminiviruses. Similarly, susceptible cabbage varieties 'Early jersey', 'California wonder' and hybrids 'King Henry' and 'Thetys' have been replaced by the resistant varieties, 'Resist crown', 'Tropicana', and 'Shael'. However, at least one of these cultivars, 'Tropicana', has been shown to be susceptible to the Jamaican isolates of CaLCuV. Plants infected with CaLCuV exhibit symptoms of yellowing and leaf curling.

In an effort to monitor the presence of whiteflies (*Bemisia tabaci*) and other vectors, a project was recently launched by USAID. Through this project farmers were provided with yellow sticky traps and greenhouses to protect plants from the whitefly vector of begomoviruses. Wherever possible, farmers maintain young seedlings under these protective greenhouse coverings, while others utilize insecticides such as Admire Pro®, Bayer, Actara®, Syngenta and Pegasus which are classified as group 4A insecticides, neonicotinoids (IRAC 2006). The mode of action of neonicotinoid pesticides is modelled after the natural insecticide, nicotine. They act on the central nervous system of insects by binding at the postsynaptic nicotinic acetylcholine receptor, resulting in accumulation of acetylcholine, leading to excitation of the nerves and eventual paralysis and death (Yamamoto *et al*. 1998; Cox 2001; Tomizawa and Casida 2003). They are not cross-resistant to the carbamate, organophosphate, or synthetic pyrethroid insecticides, which was an impetus for their development. Admire Pro® is a broad spectrum contact and locally systemic chloronicotinyl insecticide with low mammalian toxicity. It is primarily effective against aphids, whiteflies and leafhoppers (Cox 2001). Actara®, thiamethoxam, is a broad-spectrum neonicotinoid that exhibits rapid translaminar penetration (Maienfisch *et al*. 2001).

Other insecticides such as Karate, which belongs to the pyrethoid group, Newmectin which contains abamectin as well as the organic Golden Pest spray oil containing Soya bean oil are also presently available on the local market. In addition, farmers have found that applications of ReZist on seedlings shortly after transfer and establishment in the field results in decreased viral infections. It is reported that plants treated with ReZist are not as severely affected upon exposure to infectious insect vectors (Agostini 2003).

The combination of strategies employed locally in the control of geminiviruses has been successful in reducing the devastating effects on some local crops. However, the incidence of various local weeds harbouring a wide diversity of begomoviruses is of great concern. Since the majority of these begomoviruses are endemic to the island and they have not yet been characterised, their potential impact on agriculture in Jamaica is not known. Therefore, further investigations into the host range of weed begomoviruses in crop plants and their potential impact in mixed infections are imperative. Such studies will also be useful for local integrated pest management efforts.

Papaya ringspot potyvirus

Papaya ringspot potyvirus (PRSV), of the *Potyviridae*, is economically the most important virus of papaya (*Carica papaya*); a crop that is well adapted to intensive commercial orchards and the more traditional backyard stands in tropical and sub-tropical regions (Purcifull *et al.* 1984). PRSV induces a range of symptoms in papaya cultivars including vein clearing, mosaic, filiformy, ringspot blemishes on fruits, decreased yield, and stunted trees (Purcifull *et al.* 1984; Gonsalves 1998). Two pathotypes are recognized: type p isolates that are pathogenic to papaya and cucurbits and type w isolates that cause disease in cucurbits only. PRSV is transmitted mechanically and by numerous species of aphids in a non-persistent manner (Purcifull *et al.* 1984).

Although early accounts document the prevalence of PRSV in the Caribbean since 1926 (Jensen 1949), the first epidemic in Jamaica occurred in 1989 following the rapid expansion in the cultivation of solo papaya (Young 1994). Prior to 1983, there were no large scale commercial plantings of papaya in Jamaica. Large fruit types from Florida and South America were common garden plants given the popularity of papaya as breakfast or dessert fruit. Only about two hectares of commercial plantings were designated to local processing and fresh fruit markets. During the mid 1980's, however, papaya was promoted in the agricultural sector as a viable alternative to the traditional export crops of sugar cane and banana and over the next decade, 2325 hectares were established with the small "Sunrise solo" cultivar from Hawaii (Thomas 1993). Jamaica was at this time recognized as a leading exporter of papaya in the region.

In the late 1980s, diseased trees with distorted leaves and fruits with characteristic blemishes were observed in the traditional growing regions of the island, namely St. Thomas and St. Catherine. An island wide survey later confirmed that the virus was limited to these parishes. The *Papaya ringspot virus* Disease Order, 1990 under The Plants (Protection from Disease) Act was gazetted in July of the following year and officials of the Ministry of Agriculture monitored the destruction of papaya trees in the two areas and prohibited the movement of papaya seedlings (Young 1994). The 1990 Order was revoked in 1991 and replaced with another order that allowed restricted movement of seedlings across the island. Re-cultivation and export of

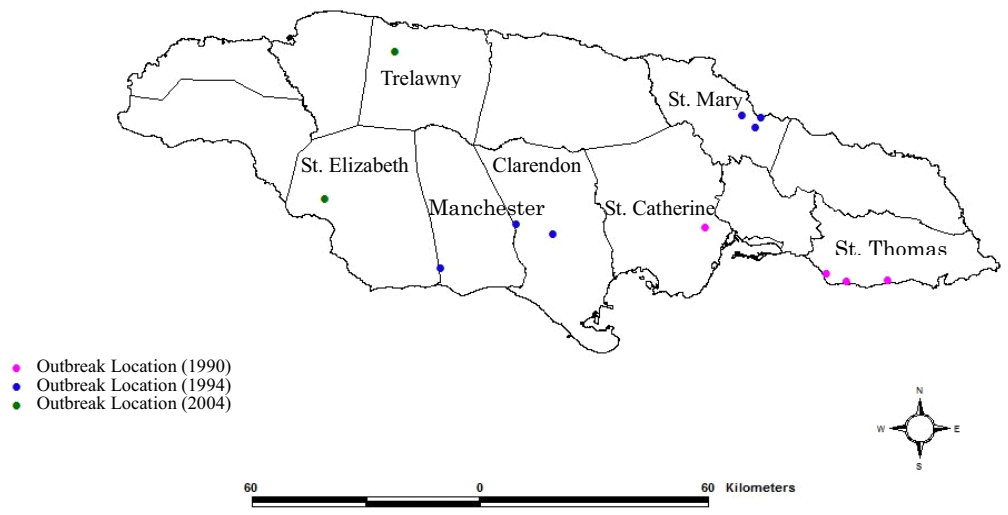

Fig. 1 Parishes affected by outbreaks of *Papaya ringspot virus* between 1990 and 1996.

Table 1 Papaya cultivation in Jamaica between 1995-2005.

Year	Papaya production (Metric tonnes)	Crop area harvested (Hectares)
1995	15,547	746
1996	12,995	572
1997	13,445	627
1998	13,700	638
1999	10,037	434
2000	8248	353
2001	8637	377
2002	9333	418
2003	9646	427
2004	7618	343
2005	9333	418

Source: FAOSTAT 2005

papaya resumed shortly thereafter since it appeared that the quick response was successful in containing the virus. However in 1994, blemished fruits destined for export were observed by Plant Quarantine officers. Field visits verified another outbreak of the virus in regions of the initial outbreak as well as in other regions of St. Mary, Clarendon and Manchester. Incidences were later reported in Trelawny in 1996 and St. Elizabeth in 2004 (**Fig. 1**). Fluctuations and the decline in papaya production over the last nine years are given in **Table 1**.

Up to this point, no formal studies on PRSV type-p strains had been done in Jamaica. However, growers in the traditional papaya growing regions of St. Thomas and St. Mary purported that PRSV isolates in these regions were more severe than in those of the recently established papaya orchards of Trelawny based on the symptom expression exhibited by field trees. Typically, PRSV infection of field trees in St. Thomas and St. Mary begins with mosaic development on the canopy followed by severe leaf distortion and discoloration of the fruits whereas infected trees in Trelawny exhibit mosaic patterns on the canopies or side shoots only after the appearance of blemishes on fruits. Serological and biological analyses with isolates from these regions were conducted in the mid 1990s. All virus isolates reacted with commercial PRSV antibody and contrary to field observations reported by local papaya growers biological tests with four indicator host plant species (*C. papaya*, *Cucumis metuliferus*, *Chenopodium quinoa* and *Nicotiana tabacum*) did not reveal any significant differences in pathogenicity between the isolates collected from the two locations (Chin *et al.* 2003).

The genetic diversity of PRSV isolates from the two regions and two time-separated outbreaks was recently examined (**Fig. 2**). The coat protein (*cp*) genes of 12 isolates were cloned and sequenced. Isolates collected from the traditional papaya growing region and the most recently established orchards in 1999 shared high similarities (average similarity values for nt and aa of 98-100% and 97-100%, respectively). Interestingly, isolates from the western region of the island showed a slightly lower variability than those from the east. However, the isolates collected in 1999 differed from an isolate collected from the traditional papaya growing region in 1990 (average similarity values for nt and aa of 92 and 91%, respectively). No recombination was found between the isolates. The differences with 1990 isolate were scattered throughout the sequences compared and most changes were located within the first quarter of the gene. As expected, the similarity increased towards the 3′ end of the *cp* sequences. The data suggested a different origin for the two outbreaks. Although early analyses of type p *cp* sequences from the USA and Australia suggested little variation among isolates of a region (Quemada *et al*. 1990; Bateson *et al*. 1994), more recent data from Mexico, Brazil, and India (Silva-Rosales *et al*. 2000; Jain *et al*. 2004) suggest greater nucleotide divergence of up to 14%. In general, variation in PRSV is accepted to be related to geographical location rather than the host range and movement in papaya as well as cucurbits (Bateson *et al.* 2002). In addition, work with type p and type w isolates in Australia provided evidence that type p isolates evolved from type w (Bateson *et al*. 1994), presumably by mutation.

In the absence of resistant varieties, growing papaya in Jamaica presently involves a combination of quarantine and cultural practices aimed at reducing sources of infection. These include restricted movement of papaya seedlings, scouting of orchards and the prompt removal of infected trees. However, the measures are effective in regions such as Trelawny, where the disease pressure is apparently low; but they are not effective in other regions of high disease pressure that are found in the traditional papaya growing regions. Cross protection was investigated shortly after the first outbreak in the early 1990s as a potential method for managing the disease. In greenhouse evaluations, "Sunrise solo" seedlings previously challenged with the mild protectant strain PRSV HA 5-1 from Hawaii exhibited symptoms after a delay of two weeks when exposed to a severe Jamaican PRSV isolate (Yeh and Gonsalves 1984; Tennant *et al.* 1994). Although symptom expression was not as severe as that on the unprotected 'Sunrise solo' seedlings, it was not likely that this method would translate to significant protection under field conditions. Moreover, given the potential disadvantages of cross protection such as the adverse effects of the protectant strain on the host, dissemination to other crops and the probability of revertants (Yeh *et al.* 1988; Bau *et al.* 2004), alternative methods of genetic resistance were more attractive and research into the development of tolerant and transgenic varieties is ongoing.

Various PRSV tolerant papaya cultivars are available in Florida – 'Cariflora' (Conover *et al.* 1986) Thailand – 'Thapra' (Prasartsee *et al.* 1995), Taiwan – 'Red Lady' and 'Known You No. 1' (Story 2002). Tolerant selections may become infected with the virus but remain symptomless or

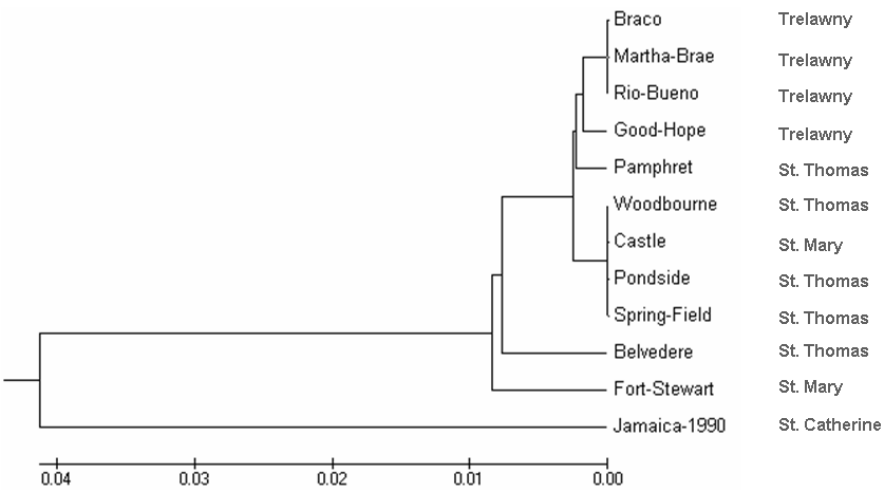

Fig. 2 Unrooted tree derived from the amino acid sequences of the *cp* genes of Jamaican PRSV isolates. The dendrogram was constructed using the neighbor-joining method with bootstrapping (1000 replicates) in MEGA after sequences were aligned with ClustalX. The locations from which the isolates were collected are indicated at the right of the dendrogram.

show mild symptom expression and produce economically useful yields (Gonsalves 1994; Zimmerman 1995). The horticultural characteristics of these tolerant selections vary from the small (0.5-0.75 kg) sweet yellow flesh fruits of 'Cariflora' to the larger (1-3 kg), light to deep yellow-fleshed fruits of 'Thapra' (Prasartsee *et al.* 1995; Gonsalves *et al*. 2005) and 'Known You No. 1', and red fleshed fruits of 'Red Lady' (Gonsalves *et al.* 2005). The reactions of tolerant varieties to PRSV isolates are also known to vary and depend on the challenge virus strain. A study was therefore initiated in the late 1990s to investigate the reactions of selected tolerant germplasm to Jamaican PRSV isolates (Turner *et al.* 2004). Three Jamaican PRSV isolates that represent the genetic and pathogenic diversity of PRSV prevalent in the island (Chin *et al.* 2003) were used for challenge inoculations.

Diverse reactions dependent on the challenge isolate and disease pressure were observed in infectivity assays under greenhouse conditions (Turner *et al*. 2004). Useful reactions of no symptoms or mild symptom expression were obtained with the *C. papaya* cultivars from Taiwan ('Red Lady'), Thailand ('Thapra') and Florida ('Cariflora'). In subsequent field evaluations diverse reactions were observed and included no foliar or fruit symptom expression, mild foliar and some fruit symptom expression and severe symptom expression on both foliage and fruits. The varieties 'Thapra' and 'Red Lady' exhibited useful levels of tolerance and good agronomic characteristics, such as good skin and acceptable brixes. On this basis, these selections are being used in a hybridization study to introduce tolerance genes in germplasm adapted for cultivation in Jamaica. Additional work into whether the agronomic performance and the size, shape, colour of the fruits of these cultivars are acceptable to the Jamaican growers or consumers is also needed.

An alternative method of managing PRSV was identified through pathogen-derived resistance involving the transfer and expression PRSV *cp* gene in transgenic papaya. Transgenic papayas were developed in a Technology Transfer Program with Cornell University and the Jamaica Agricultural Development Foundation (JADF) in the late 1990s. Papaya embryos were transformed with translatable (cp_T) and nontranslatable (cp_{NT}) versions of *cp* gene of a virus isolate from one region of the first virus outbreak (along with *npt*II and *uid*A genes) via microprojectile bombardment (Cai *et al.* 1999; Tennant *et al*. 2002). Varying levels of *cp* transcript and protein were detected in northern analysis and ELISA with cp_T lines and as expected *cp* expression was detected in cp_{NT} lines in the latter analysis (Tennant *et al*. 2002). Transgenic lines resistant to the progenitor virus were identified in greenhouse evaluation. As a result, the lines were established in the field in the late 1990s in one of the traditional papaya producing regions of the island to assess resistance under natural virus pressure. Some trees showed acceptable horticultural characteristics and exhibited a range in resistance phenotypes (Tennant *et al*. 2005). Reactions of R_0 cp_T transgenic lines ranged from asymptomatic, mild or severe leaf and fruit symptoms, or all three phenotypes in one line or between different lines. Trees of most cp_{NT} lines exhibited severe responses to infection and some also showed mild reactions. Subsequent field evaluation of the R_1 offspring was conducted and some lines showed phenotypes previously observed with parental R_0 trees, however, phenotypes not previously observed or a lower incidence of the phenotype was also obtained. It was concluded that the transgenic lines appear to possess virus disease resistance that could be manipulated in subsequent generations for the development of a product with acceptable commercial performance.

However, the genetic variation between the isolates of 1990 and 1999 raises concerns of the efficacy of sequence-based control management strategies of PRSV developed in Jamaica in the early 1990s using an isolate collected at that time. Infectivity studies with some transgenic lines carrying *cp* gene of the 1990s PRSV isolate show little resistance to challenge inoculation with isolates from St. Thomas (Belvedere) and St. Mary (Greencastle). These isolates share sequence nt similarities of 90%, suggesting that the level of resistance conferred by *cp* transgenes is not solely dependent on a high degree of similarity between the transgene and the challenge virus. Similar observations have been made with transgenic papaya developed for other regions. Fermin *et al.* (2004) reported that all R_0 transgenic lines transformed with a severe *cp* gene from Vigia (EV) Venezuela collected in 1993, were completely susceptible to mechanical challenge with PRSV EV. R_1 progenies showed 0 to 7% resistance against PRSV EV but 50-73% against LA (another Venezuelan isolate) and 50 to 60% against the Hawaiian HA isolate. Sequence similarities between the *cp* transgene and these challenge isolates were 100%, 92% and 94%, respectively. In Taiwan, transgenic papaya lines carrying the *cp* gene of the YK strain of PRSV exhibited resistance against isolates from Hawaii (HA), Mexico (MX) and Thailand (TH) but resistance was not conferred against local strains (Tripathi *et al.* 2004). Molecular characterization of the local isolates revealed high sequence similarities ranging from 95 to 96.5% to the YK strains while sequence similarities between YK and HA, MX and TH were 91.2%, 89.8%, and 92.6%, respectively. However, the control of PRSV by the use of engineered *cp* transgenes has led to the successful control of virus epidemics in Hawaii (Gonsalves 1998). The transgenic papayas carry a chimeric *cp* gene of *Cucumber mosaic virus* (CMV) and a mild strain of PRSV HA 5-1 (developed from the severe strain PRSV HA Hawaii Oahu, Yeh and Gonsalves 1984). High levels of resistance (100%) were observed in greenhouse studies against heterologous isolates, from Oahu and Panaewa, that share 96.7 to 99.8% sequence homology with *cp* transgene PRSV HA 5-1.

In Jamaica, efforts are presently focused on stabilizing, by continued self pollination, interesting lines that carry the cp_T of the PRSV isolate collected in 1990. In addition, new investigations into the efficacy of chimeric *cp* (Gonsalves and Fermin 2004) and hairpin (Wang *et al.* 2000) gene constructs are underway. Promising results have been obtained with transgenic plants carrying PRSV *cp* transgenes designed to produce hairpin RNA *in planta*. All transgenic plants carrying the hairpin construct with *cp* sequences of an isolate from St. Mary were challenge inoculated twice with the progenitor virus. Plants did not develop symptoms and have been maintained in the greenhouse for over three months without break down in resistance. Other plants were inoculated with virus from St. Catherine and Trelawny and similar reactions were obtained.

Although much of the basic research necessary for the development of transgenic papaya has already been undertaken, two challenges to commercialization remain and are associated with deregulation and public acceptance. Even though Jamaica established a National Biosafety Committee in the late 1990s and initiated field testing of genetically modified plants in 1998, the regulatory guidelines for the release of genetically modified organisms have not been formulated. Nevertheless, some growers have displayed a keen interest in understanding the technology and have visited the field trials and identified transgenic trees exhibiting acceptable commercial traits. However, others are hesitant of the introduction of the material into commerce. This is because their major markets are in Europe and they fear genetic contamination of their non-transformed materials. Others question the safety of the genetically modified product. Therefore, the challenge lies in satisfying the country's regulations, the market, farmer, and consumer. Perhaps a combination of conventional and technologically based approaches is the most promising for managing PRSV in Jamaica.

Citrus tristeza closterovirus

Citrus tristeza virus (CTV) is one of the most severe pathogens of citrus worldwide. The virus belongs to the genus

Closterovirus within the *Closteroviridae* family. *Citrus tristeza virus*, a phloem-limited virus, is transmitted semi-persistently by seven aphid species (Brlansky *et al*. 1988) as well as through the use of contaminated budwood in the citrus grafting process. The virus is genetically and biologically diverse; causing stunting, slow decline, quick decline, stem pitting, or no expression of symptoms depending on the virus isolate, rootstock, citrus cultivar, and environmental conditions (Garnsey *et al*. 1987). For the farmer, invasion of CTV into the citrus orchard translates to eventual reduction in fruit size, quality and yield and or quick decline leading to tree death.

Although CTV was first detected in Jamaica in 1959 (Lastra *et al*. 1992; Yokomi *et al*. 1994), the pathogen currently poses a threat to the citrus industry since the severe strain of CTV and one of its most efficient vectors, *Toxoptera citricida,* were recently confirmed in Jamaica (Edman and Young 1998, 1999; Lee *et al*. 2003). All citrus varieties grown in Jamaica are susceptible to CTV. These include sweet orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*), lime (*Citrus aurantifolia*), *Ortanique* (*Citrus* × *nobilis*), a unique Jamaican hybrid fruit and Ugli® (*C. reticulata* × *C. paradisi*) a cross between an orange and a tangerine that peels easily and is about twice the size of a regular orange. Furthermore, the principal rootstock used in the industry is sour orange ('Bitter sweet Seville' and some shaddock rootstocks) which is also very susceptible to the virus (Lee *et al.* 2003). In the last 13 years, Jamaican farmers have more than doubled the acreage of citrus production. The major citrus growing regions or parishes in Jamaica include Clarendon, Manchester, St. Catherine, St. James, and St. Mary but farms ranging in 0.2 to 8 hectares can be found in all 14 parishes across the island. Seventy percent of the citrus produced goes to the United Kingdom and 30% is exported to the United States, Canada and neighboring Caribbean islands. It is, however, feared that the industry could be wiped out by CTV within the next few years. Recent figures out of the Planning Institute of Jamaica's Economic and Social Survey show that the value of Jamaica's fresh citrus fruit exports fell from US$ 4.032 million in 2001 to US$ 1.48 million in 2005. Of even equal importance is the loss of the livelihood of many small scale farmers in a substantial area of St. Catherine, Clarendon and some areas in Manchester.

Efforts to determine the distribution of CTV and develop certification schemes were initiated in 1999. With assistance from the United Nations' Food and Agricultural Organization (FAO) a project was implemented and the first systematic survey of commercial citrus acreage was conducted in 1999 (Edman and Young 1999). Using ELISA with polyclonal antibodies to CTV strains, virus was detected in all parishes with incidences ranging from 3% to 80% in 13 regions. Detection of CTV in the major citrus growing regions was 4% in Clarendon, 9-12% in St. Mary, Manchester and St. James, 38% in Hanover and 80% in St. Catherine. The assessment not only included testing for the presence or absence of CTV, but also involved the determination of severe CTV strains using monoclonal antibodies. Lower incidences (1-22%) of severe CTV strains were obtained with samples from 10 regions. Detection of severe CTV in the major citrus growing regions was 1% in Clarendon, 2-8% in St. Mary, St. Catherine, Manchester, and St. James, and 22% in Hanover. A five year Citrus Replanting project involving the removal and replanting of 2,833 hectares of groves and the formulation of mandatory certification were instituted in anticipation of losses on sour orange rootstock due to CTV.

Additional support for the project was obtained in 2001 from the Caribbean Development Bank, the Government of Jamaica through the Development Bank of Jamaica, the Citrus Growers' Association and the Jamaica Citrus Protection Agency. This allowed for the provision of farmers with loans for undertaking the replanting of orchards, technical assistance and a public education component. Other activities under the project included an investigation of the disease on a parish by parish basis and monitoring the production and tagging of certified planting materials at nurseries.

CTV-tolerant rootstocks for replanting were imported from California and South America. These included some 14 varieties such as 'Swingle citrumelo', 'Cleopatra mandarin', 'Smooth Flat Swingle', 'Gau tau', 'Carrizo citrange', 'Volkameriana' and 'Rangpur Lime'. The certified propagation, at three locations, was established under greenhouse conditions from which quick multiplication of planting materials was conducted. Mainly five varieties ('Cleopatra mandarin', 'Swingle citrumelo', 'Smooth Flat Swingle', 'Gau Tau' and 'Carrizo citrange') are being propagated and distributed by registered nurseries to farmers. Although initial agronomic testing was not performed with the imported rootstocks prior to the multiplication and distribution, it appears that some have fared well against the soil types of many citrus growing regions. Recent investigations on farmers' orchards show that 'Cleopatra mandarin' performs well on calcareous soils and 'Swingle citrumelo' rootstock is favored for strongly acid to moderate acid clay.

The Citrus Replanting Project should have been completed in October 2006. However, about a third of the targeted acreage was replanted with certified planting material tolerant to the virus with funding either through project loans or by funds of individual farmers. The appeal to farmers continues; that is a public education program to heighten awareness of the effects of the disease on the citrus industry and the need to accelerate replanting with certified planting material. The farmers have raised a number of factors that have contributed to the slow progress of replanting. These mainly surround the processing and procedure of the disbursement of loans. In general, however, small scale farmers are known to be risk adverse and many have not taken up the loans and some have started to finance the replanting on their own. Given this, an assistance program was formulated to assist farmers who through their own initiative have started replanting using their own funds. These farmers are to receive certified planting materials along with chemicals for fertilization and pest control. The project had been extended until March 2007 (Ministry of Agriculture 2006).

The slow progress in replanting is particularly worrisome given the results of the recent survey of the citrus growing regions in 2004. One thousand eight hundred and eighty five (1,885) samples were analysed for CTV strains using ELISA. CTV was detected in all parishes. The incidences ranged from 7% to 100%. Ten parishes had CTV incidences over 50%. The highest CTV incidences were recorded in the parishes St. Mary (85%) and St. Catherine (100%). Furthermore, severe CTV strains were detected in nine parishes with incidences up to 33%. The highest incidence of the severe CTV strain was obtained in samples from St. Catherine (33%). No severe CTV strains were detected in Portland, St. Thomas, Trelawny and Westmoreland. Given the results of the survey conducted in 1999 (Edman and Young 1999), the incidence of CTV has increased in all parishes except St. Ann. The incidence of severe CTV isolates increased in Clarendon, Manchester, St. Catherine, St. Mary, St. Elizabeth, and St. Andrew. Severe CTV strain was not previously reported in St. Andrew. There was a decrease in the incidence of severe CTV strains in Trelawny, Westmoreland, St. James, Hanover and St. Ann and the incidence remained the same in two parishes since 1999 (**Table 2**).

About a sixth of the samples collected were analysed for CTV strains using reverse transcriptase polymerase chain reaction (RT-PCR) with oligonucleotide primers C74-1 and C100-1 (Mehta *et al*. 1997) specific to the coat protein gene of a decline strain of CTV from Florida. CTV detection with primers C74-1 and C100-1 ranged from **6%** to 54% in eight parishes. Comparable results were not obtained in the RT-PCR and ELISA tests. CTV was detected at higher levels in some parishes (e.g. Hanover) using RT-PCR when compared with ELISA. The converse was also

Table 2 Comparative data of CTV incidence in 1999 and 2004 in fourteen parishes in Jamaica.

Parish	ELISA: Broad Spectrum Detection				ELISA: Severe Detection			
	n	% incidence 1999[a]	n	% incidence 2004	n	% incidence 1999[a]	n	% incidence 2004
Clarendon	1373	4	494	68	1373	1	494	14
Hanover	322	38	87	78	322	22	87	6
Manchester	304	12	123	61	304	5.4	123	26
Portland	127	3	37	24	127	0	37	0
St. Andrew	50	6	24	63	50	0	24	8
St. Ann	229	36	74	7	229	20	74	5
St. Catherine	1352	80	577	100	1352	8	577	33
St. Elizabeth	166	6	24	54	166	1.5	24	29
St. James	203	12	87	52	203	4.2	87	2
St. Mary	816	9	236	85	816	2.3	236	24
St. Thomas	26	21	12	67	26	0	12	0
Trelawny	134	13	62	53	134	2.6	62	0
Westmoreland	164	15	48	40	164	10	48	0

[a]Source: Edman and Young 1999

Table 3 Detection and differentiation of *Citrus tristeza virus* (CTV) in Jamaica using ELISA and RT-PCR.

Parish	ELISA[a]				RT-PCR[b]		
	n_{farms}	$n_{samples}$	% incidence CTV	% incidence severe CTV	n_{farms}	$n_{samples}$	% incidence CTV
Clarendon	18	494	68	14	2	22	50
Hanover	3	87	77	5	1	10	50
Manchester	6	123	61	26	1	10	0
Portland	2	37	24	0	1	12	0
St. Andrew	2	24	63	8	2	8	13
St. Ann	3	74	7	5	3	32	6
St. Catherine	16	577	100	33	7	160	54
St. Elizabeth	2	24	54	29	1	10	0
St. James	3	87	52	2	1	10	10
St. Mary	8	236	85	24	1	10	30
St. Thomas	1	12	67	0	1	10	0
Trelawny	2	62	53	0	1	10	10
Westmoreland	3	48	40	0	1	10	0

[a] n_{farms} number of farms sampled, $n_{samples}$ number of samples tested; negative controls in ELISA for the detection of CTV gave OD_{405nm} readings of 0.011-0.042 and positive controls 1.621 to 1.974. In ELISA for the detection of the severe CTV; negative controls gave OD_{405nm} readings of -0.017 to 0.042 and positive controls 0.109 to 0.715. % incidence determined as percentage of the total number of positive samples obtained out of the total number of samples tested.

[b] based on the amplification of the CTV coat protein gene of about 670 bp; % incidence determined as percentage of the total number of positive samples obtained out of the total number of samples tested.

observed (e.g. St. Elizabeth). However, the data from both tests suggest that severe CTV is not present in some locations of Manchester, St. James, St. Elizabeth, Portland, St. Thomas, and St. Andrew (**Table 3**).

Molecular characterization of CTV strains from two commercial citrus growing regions of Jamaica was recently started using samples established on Mexican lime, sweet orange or sweet orange on sour orange. The coat protein genes (670 bp) of two mild and three severe CTV strains isolated from St. Catherine and Clarendon were cloned and sequenced. High nucleotide and amino acid similarities were obtained between the mild and severe CTV isolates from both regions. However, both mild isolates shared higher nucleotide similarities with the severe isolates for the respective regions (93-96%). Similarly, the stem pitting isolate had lower nucleotide similarities with the mild isolates. The stem pitting isolate from St. Catherine had nucleotide sequence similarities of 96% and 97% with the severe isolates from St. Catherine and Clarendon, respectively. The stem pitting isolate from St. Catherine had nucleotide sequence similarities of 93% to the mild isolates (**Table 4**). The differences between the stem pitting isolate and the mild and severe isolates were scattered throughout the genes and not clustered to any region.

When aligned sequences were viewed as a phylogenetic tree (**Fig. 3**), the relationships among the isolates suggested the existence of three clades. One clade contained the mild isolate from St. Catherine, another, the severe isolate from Clarendon and the third both the severe and stem pitting isolates from St. Catherine along with the mild isolate from Clarendon. This variation between isolates and closer similarity between other isolates has been reported with CTV in other regions and is attributed to interactions in mixed infections (Rubio *et al*. 2001; d'Urso *et al*. 2003). It is also documented that CTV populations, as with other RNA viruses, consist of different sequence variants the composition of which is altered by graft and vector transmission (Albiach-Marti *et al*. 2000; d'Urso *et al*. 2000; Ayllon *et al*. 2006) and introductions (Cambra *et al*. 2000; Alvai *et al*. 2005). Similar to previous studies in Florida and Spain (Permar *et al*. 1990; Rubio *et al*. 2001) and unlike studies in India and Iran (Roy *et al*. 2003; Alavi *et al*. 2005), the data

Table 4 Percent coat protein nucleotide and amino acid sequence similarities between mild and severe Jamaican CTV isolates.

					← % Nucleotide similarity
Isolates	Clarendon_mild	Clarendon_severe	St. Catherine_mild	St. Catherine_severe	St. Catherine_pitting
Clarendon_mild	–	94	89	96	93
Clarendon_severe	89	–	93	95	97
St. Catherine_mild	86	91	–	91	93
St. Catherine_severe	89	91	87	–	96
St. Catherine_pitting	89	93	89	97	–
	% Amino acid similarity →				

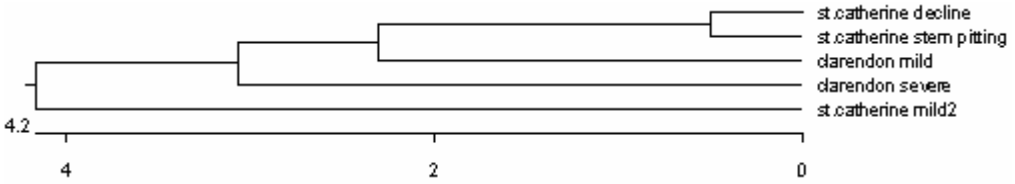

Fig. 3 Phylogenetic tree from amino acid sequences of the coat protein gene of CTV isolates from Jamaica.

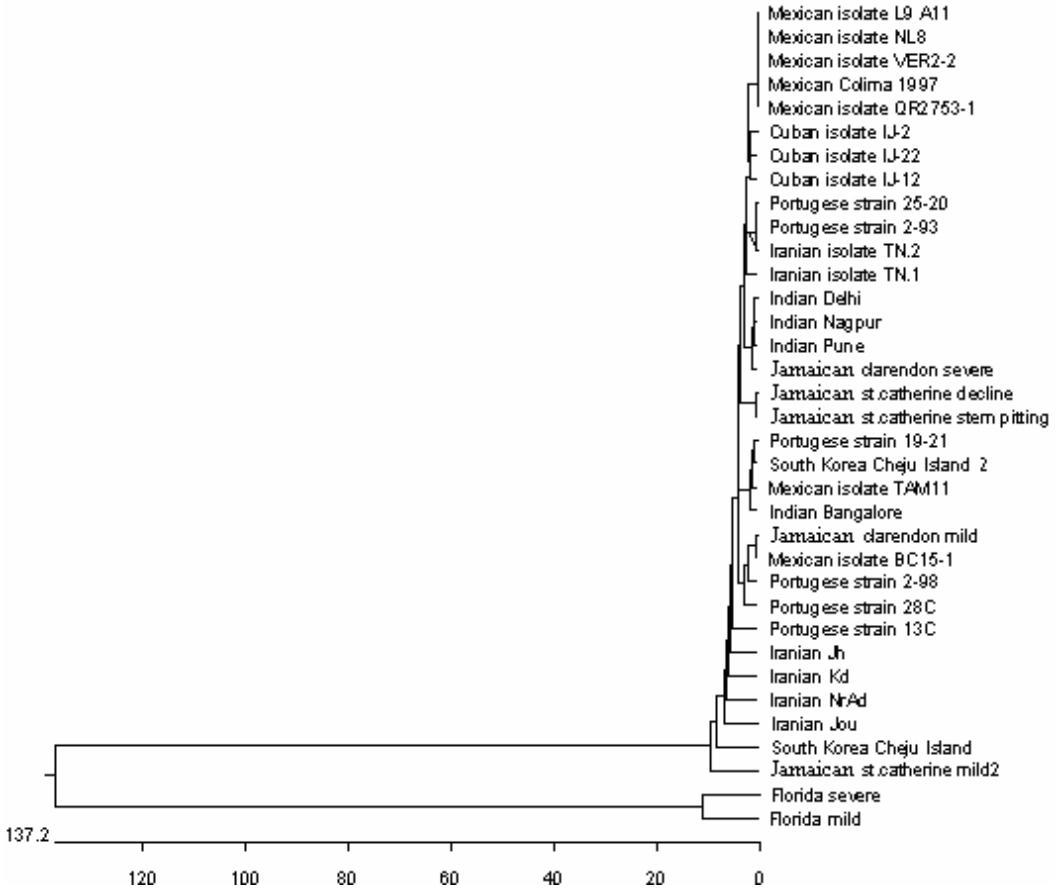

Fig. 4 Phylogenetic tree from amino acid sequences of the coat protein gene of CTV isolates from Jamaica and the other regions of the world. The latter sequences are published sequences from Florida (AF260651 Mild, AY170468 Severe), South Korea (Cheju Island AF249279, isolate 2 AF339088), Iran (Jou AY803274, NrAd AY803276, Kd AY803278, Jh AY803277, TN.1 AY707459, TN.2 AY707460), Portugal (13C AF184113, 28C AF184118, 2-98 AF184117, 19-21 AF184114, 2-93 AF184116, 25-20 AF184115), Mexico (BC15-1 AF342894, TAM11 AF342890, QR2753-1 AF342893, Colima 1997 AY652894, VER2-2 AF342892, NL8 AF342891, L9 A11 AF342895), India (Bangalore AF501867, Pune AF501869, Nagpur AF501870, Dehli AF501868) and Cuba (IJ-12 AF220503, IJ-22 AF220504, IJ-2 AF220502).

suggest that strains of CTV with coat protein gene sequences that are very similar can exhibit different biological properties. However, the study on the genetic diversity of CTV strains in Jamaica is not complete. The strains of CTV from Jamaica need to be further characterized for the development of a more robust ELISA and RT-PCR detection assay that will allow for the detection of most if not all the isolates. This will invariably lead to an understanding of the forces driving the diversity in Jamaica and assist in monitoring the disease and in the application of effective control measures.

Comparisons were also done with the Jamaican isolates and CTV isolates from other citrus growing regions worldwide. Two clades were observed in this analysis; one with the isolates from Florida and the other contained the Jamaican isolates and isolates from the Caribbean, the Americas and the East. Interestingly, the Jamaican CTV isolates did not all cluster together but with either Mexican, Portuguese, Indian isolates or isolates from Florida (**Fig. 4**).

Concurrent to the characterization of CTV isolates is the development of other methods for the provision of certified clean planting materials. Protocols are being developed for shoot tip grafting of local citrus varieties onto tolerant rootstocks (Francis *et al*. unpublished data). Shoot tip grafting is a well established technique used in the elimination of virus pathogens from citrus germplasm (Murashige *et al*. 1972; Navarro *et al*. 1975; Edriss and Burger 1984; Starrantino and Caruso 1988; Navarro 1992; Kumarin and Singh 2000). The method involves micro-grafting of aseptically isolated shoot tips to *in vitro* grown virus free rootstock seedlings. Once the graft has taken, the seedlings are transferred to the greenhouse and subsequently tested to ensure that all pathogens previously detected in the materials used to generate the shoot tips have been successfully removed. The micro-grafts can then be used as a source of budwood for propagation.

During the survey of CTV across the island, a number of healthy-looking trees in infected orchards were observed and later found to be carrying both mild and severe CTV strains. Similar evidence of cross protection among some CTV strains has been observed in mixed infections and reported an effective CTV management strategy in regions such as Australia, Brazil, India, South Africa, Florida, and Japan (Costa and Muller 1980; Bar-Joseph *et. al.* 1989; van Vuuren *et al*. 1993; Fuchs *et al*. 1997; Powell *et al*. 1999). Cross protection is defined as the use of a mild strain of a virus to protect against a more severe strain of the virus (Gonsalves and Garnsey 1989; Rocha-Pena *et al.* 1995). Severe symptom expression is often reduced and the life expectancy of trees extended. However, not enough is known about strain severity and diversity, or of the combinations of the Jamaican CTV strains, to adequately evaluate the benefit or risk of the cross protection strategy to the industry at this time. Evaluation of specific host effects and protecting abilities of the mild strains is required given that CTV strains are defined by their reaction on various citrus host combinations and environmental conditions. The usefulness of the procedure for the Jamaican industry will be realized after these screenings and the characterization of the genetic groups of CTV across the island. Until then management of the disease continues to involve tree monitoring for CTV followed with mandatory removal of infec-

ted trees and replanting on tolerant rootstocks based on current data that severe strains are already present in Jamaica, that others may appear and the assumption that mild strains infecting trees on tolerant rootstocks may produce smaller yields.

Genetic resistance is the ideal method of controlling CTV. Despite genetic diversity and inter-specific fertility in the genus *Citrus*, breeding is difficult and few varieties are available (Moore *et al.* 1993; Gmitter *et al*. 1996). As a result, several research groups have examined pathogen derived-resistance and direct genetic manipulation of citrus for the development of post transcriptional gene silencing (PTGS) against CTV. Varying levels of resistance have been reported with transgenic plants (*Citrus aurantifolia* [Christ.] Swing.) carrying the capsid protein *p25* following exposure to virus via aphid and graft inoculations. The phenotypes included no symptom expression, or delay in symptom expression and virus accumulation. Resistance was also obtained against non-homologous strains (Dominguez *et al.* 2002). However, other transformation experiments with citrus (*Citrus aurantifolia* [Christ.] Swing.) transgenic for the silencing suppressor and pathogenicity determinant, *p23* (Lu *et al.* 2004) exhibited developmental abnormalities closely resembling virus-like symptoms (Fagoaga *et al*. 2005). Subsequent studies with similarly transformed citrus showing normal phenotypes exhibited strong levels of resistance against CTV (Fagoaga *et al.* 2006). Transgenic plants that produce dsRNA transcripts of *p23* transcripts did not provide resistance against CTV (Batuman *et al.* 2006). Given that CTV has a sophisticated three gene (*p20*, *p23*, *p25*) counter-defense strategy against RNA silencing (Lu *et al.* 2004); perhaps a transgene designed with all three genes may be useful in the development of transgenic resistance against CTV. Whether transgenic plants could be an alternative strategy for managing CTV in the field remains to be tested. Moreover, the application of these control measures, transgenic resistance as well as cross-protection, should be considered with caution in light of the frequency of recombination (Rubio *et al*. 2001; Vives *et al*. 2005) among CTV isolates. Introduction of any exotic sequences might facilitate the development and establishment of isolates with new biological properties.

Taken together, tristeza has changed the traditional methods of growing citrus in Jamaica. The industry is slowly heading towards solving the spread of tristeza through adopting a zero CTV tolerance in nursery plants and replanting with tolerant rootstocks. While this is an immediate solution, research must continue to develop appropriate tools for controlling future outbreaks either from the emergence of new CTV strains or from the introduction of new strains, as has been reported in other citrus growing regions.

CONCLUDING REMARKS

Viruses of the *Geminiviridae*, *Potyviridae*, and *Closteroviridae* have caused severe epidemics in the past decade in Jamaica. Factors contributing to these epidemics differ but generally involve the interplay of crop intensification, host range extension, virus species interactions, dissemination of the virus, and increasing traffic in plants and plant products. Various management strategies reported useful in developed countries have been tailored for adoption in Jamaica. However, success in implementation varies and is mainly influenced by the farmers' perceived associated risk, their reaction to changes in the cropping system, social and economic factors and the amount of assistance extended to farmers. Above all efforts in managing plant virus diseases in the region require additional investigations on not only the pathogens but also on their epidemiology and control.

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