Papaya Ring Spot, Banana Bunchy Top, and Citrus Greening in the Asia and Pacific Region: Occurrence and Control Strategy

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Abstract

Results obtained in a special project on insect-borne virus/virus-like diseases of tropical fruit trees implemented during the period 1991-1995 are presented. Incidence of papaya ring spot virus (PRSV), papaya leaf-distortion mosaic virus (PLDMV), banana bunchy top virus (BBTV), cucumber mosaic virus (CMV), citrus greening and citrus tristeza virus (CTV) was examined by ELISA tests for virus diseases of papaya, banana and citrus, and by DNA probes for citrus greening. The FFTC survey teams visited countries in the Asia and Pacific region including Fiji, Guam, Indonesia, Japan (Okinawa), Korea (Cheju), Mainland China, Peninsular Malaysia (West Malaysia), Sabah and Sarawak (East Malaysia), New Caledonia, Palau, the Philippines, Pohnpei, Saipan, Taiwan, Thailand, Tonga, Vietnam and Western Samoa. The distribution of PRSV and PLDMV diseases is expanding. A large area covering East Malaysia, Mindanao, Fiji, Indonesia, New Caledonia, Palau, Pohnpei, Tonga, Western Samoa was found to be free from these diseases. In Okinawa, Taiwan and Saipan which are located in the northern marginal area of distribution of PRSV, the incidence of PLDMV was also observed. BBTV used to occur together with CMV. BBTV had a wider distribution than had been previously assumed, because banana plants did not show lesions when infected with the symptomless mild strain of BBTV. Except for some Pacific islands including those in Micronesia and New Caledonia, all the countries visited were found to be infected with both BBTV and CMV. Citrus was affected by the greening disease and CTV except for some islands in Micronesia. The distribution of a new greening strain which infects pomelo is now expanding. In addition to Taiwan and Mainland China, Okinawa, Mindanao, Palau and Thailand, citrus trees including pomelo are likely to be destroyed unless appropriate control measures are taken. Integrated management system of insect-borne diseases of fruit trees was analyzed in terms of the development of a production system for virus-free seedlings, protection of seedlings from attacks of viruliferous vectors and the elimination of virus sources.

Discipline: Plant disease/Insect pest **Additional key words:** insect-borne diseases, IPM, fruit tree, aphids, citrus psyllids

Introduction

The Food and Fertilizer Technology Center for the Asian and Pacific Region (FFTC/ASPAC) conducted a Special Project on insect-borne virus (-like) diseases in the tropics and subtropics. This project was funded by the Japanese Government, but some activities conducted in Taiwan were supported by the Council of Agriculture (COA), Taiwan.

Emphasis was placed on fruit trees, because there is no international research institute dealing with tropical fruits except for banana, and because the control of insect-borne diseases requires interdisciplinary cooperation between e.g. entomologists and virologists. Furthermore, perennial crops such as fruit trees

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are likely to be exposed for a relatively long period of time to infection with viruses, making the control of the diseases extremely difficult.

The FFTC organized survey teams, composed of entomologists and virologists, on insect-borne diseases of citrus, papaya and banana to be conducted in countries of the Asia and Pacific region. Since 1988, when the first survey was conducted in the Philippines and Thailand, survey teams have visited many countries in the Asia and Pacific region, including Fiji, Malaysia (East and West), New Caledonia, Palau, the Philippines, Pohnpei, Saipan, Taiwan, Thailand, Tonga, Vietnam and Western Samoa.

The incidence of fruit tree diseases in the Asia and Pacific region is reported in this paper, i.e. diseases caused by banana bunchy top virus (BBTV), papaya ring spot virus (PRSV), papaya leaf distortion mosaic virus (PLDMV), citrus tristeza virus (CTV) and greening (GO), and strategy for the application of IPM for the control of these diseases is also outlined.

Papaya ring spot virus (PRSV) and papaya leafdistortion mosaic virus (PLDMV) diseases

1) Expansion of papaya virus diseases

PRSV disease was first detected in Kaoshung,

Taiwan in 1975, and in Luzon, the Philippines in 1982. In Taiwan, 44% of a total of 2,100 ha of papaya orchards were not able to produce fruits in 1978 due to PRSV disease, which spread all over the island in 1980¹⁵⁾.

The FFTC survey team confirmed the incidence of PRSV disease in Johore, Malaysia based on serological diagnosis in 1991. We also confirmed the occurrence of PRSV disease in Southern China (1990), Central Thailand (1990), Vietnam (1992), Luzon, the Philippines (1993), Saipan, Commonwealth of the Northern Marianas (1994) and Guam (1994). No infection had been detected in Northern Thailand, Mindanao and Cebu (Philippines), Sabah and Sarawak (East Malaysia), Java (Indonesia), New Caledonia, Fiji, Western Samoa, Tonga, Palau and Pohnpei (Table 1).

Papaya trees infected with PLDMV display symptoms characterized by rosettes on leaves with slender stems on the crown top, and swellings around the ring spots on the fruit. Neither of these symptoms are observed in plants infected with PRSV. PLDMV disease was first identified in Okinawa island in 1954¹⁰. During the 1960s it spread to the adjacent Miyako and Yaeyama islands, but it has not yet been detected in nearby Minami-Daito or Kita-Daito island¹⁰. In 1993, collaborative studies between

A	Papaya		Banana		Citrus	
Country / locality	PRSV	PLDMV	BBTV	CMV	GO	CTV
Fiji			0	0	0	0
Guam	0		2003	2000	NE	NE
Indonesia / Java		-	0	NE	0	0
Japan / Okinawa	0	0	0	0	0	0
Korea / Cheju		9 -0 1			3 	0
Mainland China / South	0		0	NE	0	0
Malaysia / West	0		0	0	0	0
Malaysia / Sabah			0	NE	0	NE
Malaysia / Sarawak		-	0	NE	0	NE
New Caledonia	-			NE	_	-
Palau	-		-		0	
Philippines / Luzon	0	-	0	NE	0	0
Philippines / Cebu	877	-	0	NE	0	0
Philippines / Mindanao			0	0	0	0
Pohnpei		-	-	NE	-	-
Saipan	0	0				
Taiwan	0	0	0	0	Ø	0
Thailand / North	·		0	0	Ø	0
Thailand / Central	0		0	0	0	0
Tonga	-		0	0	0	0
Vietnam	0		0	0	0	0
Western Samoa			0	0	0	0

Table 1. Distribution of virus(-like) diseases of fruit trees in the Asia and Pacific region

GO: Greening, -: Absence, O: Presence, @: Strain attacking pomelo, NE: Not examined.

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	PRSV	BBTV	Greening
Host plant	Papaya	Banana	Citrus
Country of origin	South America	S. E. Asia	Asia
Resistance gene/cultivar	No	No	No
Cropping system	Annual/perennial	Annual/perennial	Perennial
Disease-free planting material	Seedling	Tissue culture of meristem	Shoot-tip grafting
Pathogen	Virus	Virus	Fastidious bacteria
Culture of inoculum	Possible	Possible	Impossible
Indexing techniques	ELISA	ELISA	DNA probe
Transmission type	Non-persistent	Semi-persistent	Persistent
Cross-protection	Not dependable	Not dependable	Not available
Chemical therapy	No	No	Antibiotics
Vector	Polyphagous aphids	Banana aphid	Citrus psyllid
Colonization of host plants	No, transit visitors as winged forms	Yes, attended by ants	Yes
Biological control	Difficult in open fields	Failed (interference by ants)	Not successful (hyperparasitism)
Chemical control	Difficult to control disease vectors with a low control threshold		

Table 2.	Characteristics of papaya ring spot virus (PRSV), banana bunchy top virus (BBTV),
	and citrus greening diseases

scientists in Taiwan and Japan confirmed that these 2 virus diseases occurred in both Okinawa and Taiwan, i.e. PLDMV also occurs in Taiwan, and PRSV in Okinawa¹⁶⁾. However, the symptoms of PLDMV had not been observed in Taiwan until the mid-1980s.

2) Control of papaya virus diseases

More than 60 species of aphids are considered to be able to transmit PRSV. Both PRSV and PLDMV are mainly transmitted by *Aphis gossypii* and *Myzus persicae* in a non-persistent manner. They are the "transit visitors or itinerant vectors" of papaya plants and do not colonize papaya (Table 2). These virus diseases are currently spreading throughout the Asian tropics and Pacific islands.

A great variety of control methods has been used in order to protect young seedlings from the aphid vectors. These include the selection of planting time to avoid the peak incidence of winged aphids, intercropping with barrier crops such as corn, the use of silver mulch to prevent winged aphids from visiting young seedlings, and covering the plants with netting.

A basic part of any control program is to promote the eradication of infected plants in order to eliminate the source of virus. The cultivation of cucurbits, which are important hosts for PRSV and PLDMV should be avoided in the vicinity of papaya plantations. Once PRSV and PLDMV diseases occurred in a papaya orchard, they spread very rapidly within the orchard by winged aphids, usually in February and March in Taiwan¹⁴⁾, and December to April in Okinawa¹⁰⁾. Roguing infected plants away from the orchard is effective to reduce the rate of infection.

Since there is no PRSV resistance gene, the breeding of tolerant cultivars such as Taichung No. 5 has been attempted. These varieties, however, have not been widely accepted by consumers because of their poor eating quality.

Difficulty encountered in controlling non-persistent aphid-borne virus like PRSV is due to the fact that many of the available pesticides do not kill incoming vectors before they transmit the virus to the crop.

Cross-protection with attenuated virus strains was widely practiced in Taiwan. Since this protection is strain-specific, it often breaks down within half a year and loses its effectiveness. The high level of mutability of PRSV might be another reason for the loss of cross-protection¹⁴.

3) Losses

Concerning the losses due to PRSV, the occurrence of the disease was compared between papaya orchards in Fengshan, Taiwan for which netting was used and those left uncovered. Netting was so effective in preventing PRSV transmission that the production was 10 times higher compared with that of the uncovered trees¹⁵⁾. In Taiwan, papaya is planted each year as if it were an annual crop. Therefore, protecting young papaya plants from vector aphids by netting is effective in producing marketable fruits, because late infection, which occurs when the net is removed after fruiting, causes little damage to the yield. The economic gains, however, have to be evaluated taking into account the cost of building the structure, labor-consuming operation for pollination and the risk of damage caused by typhoons. Another added cost is the annual planting of papaya, which used to be a perennial crop grown for 3-4years before 1975, when the incidence of PRSV was first observed in Taiwan.

The areas where papaya is free from these virus disease should be protected by restricting the importation of infected plants. These viruses, however, are not vertically transmitted through seeds. Since the seeds of papaya are free from virus, they can be used in plant nurseries without risk of infection.

Banana bunchy top virus (BBTV) disease

 Ubiquitous potential infection with mild strains BBTV is transmitted semi-persistently by a single vector, the banana aphid, *Pentalonia nigronervosa* (Table 2). Sometimes the damage caused by BBTV disease is overlooked due to the apparent losses due to Fusarium wilt and Sigatoka diseases, and banana plants are considered to be free from BBTV.

However, the FFTC surveys demonstrated that BBTV has a wider distribution than had previously been assumed. BBTV is widespread in tropical and subtropical Asia (Table 1). It was first reported in Fiji in 1889 and Taiwan in 190019). It was also reported in Okinawa, Japan by Nohara in 1968 and was serologically confirmed by the FFTC survey team in 1992¹¹⁾. In almost all of the countries except for the islands located in Micronesia, banana plants were found to be infected with BBTV. The existence of BBTV was newly confirmed by ELISA tests in Indonesia (1989), Thailand (1991), West Malaysia (1991), South China (1992), Okinawa (1992), Vietnam (1992), East Malaysia (1993), Mindanao, the Philippines (1993), Fiji (1994), Tonga (1994) and Western Samoa (1994). On the other hand, we have not found BBTV in Micronesian islands including Saipan/Northern Marianas, Guam, Belau/Palau, and Pohnpei.

Why has BBTV remained undetected for so long in so many countries? There are 2 main reasons for this. First, no serological tests had ever been attempted, because of the lack of suitable techniques and equipment for indexing. Secondly, BBTV occurring in Malaysia and Thailand produces either mild symptoms or no symptoms in banana plants. An overall abnormality such as dwarf growth habit caused by infection with the virus has often been mistaken for a normal characteristic of the cultivar or for a nutritional disorder due to deficiency of nutrients.

Although a certain level of cross-protection with naturally occurring mild strains takes place inadvertently, this latent infection with BBTV is probably causing significant losses in terms of both banana yield and general fruit quality.

A field survey using ELISA tests for banana plants which was conducted in several locations in southern Taiwan revealed that mixed infection with BBTV and cucumber mosaic virus (CMV) was observed at a rate as high as 70% in symptomless plants²³⁾. Asynchronized maturation of fingers in a bunch of bananas grown for export seems to be caused by a mixed latent infection of the viruses. Almost the same situation was also observed in the South Pacific islands including Fiji, Western Samoa and Tonga, in that only one-fifth of the banana samples was free from both virus diseases. Imada et al.⁸⁾, based on ELISA tests, showed that both BBTV and CMV are widely distributed in Thailand and samples with double infection accounted for more than 10% of the total.

2) Control of BBTV

To control BBTV disease, a system of virus-free seedling production is highly recommended (Table 2). The occurrence of BBTV was the lowest in plants produced by tissue culture at 0.2% followed in increasing order by plants grown from suckers and ratoons (Su et al. unpublished).

No plant resistance to BBTV has been identified, which limits conventional breeding approaches to this problem⁹⁾. In fact, propagation of virus-free seedlings by tissue culture is commonly attempted in almost all the banana-growing countries. In some cases, however, all of the seedlings assumed to be virus-free were found by ELISA tests to be infected with BBTV. Supplying virus-free plantlets grown by tissue culture to growers for planting is the best measure currently available for control.

Various methods of protection of seedlings from infection in the field should then be applied, including cross-protection, though this technique has not been effective against BBTV. In order to promote the control of the banana aphid by naturally occurring biological control agents, farmers should be instructed to use in their banana plantations selective insecticides, which are less toxic to natural enemies of banana aphids.

Biological control of *P. nigronervosa* is being attempted in Tonga introducing a braconid, *Aphidius colemani*, native to Australia. Through the reduction of the total population of aphids, a reduction in the number of alate aphids moving between plants may occur, resulting in a decrease of the rate of spread of the BBTV^{21,22)}. Although the presence of *A. colemani* on Tongatapu island was confirmed when it was recovered from *Aphis gossipii* on taro, no evidence of parasitism has been obtained from *P. nigronervosa*. The intensive attendance of *P. nigronervosa* colonies by ants may have significantly reduced the opportunity for this parasitoid to attack *P. nigronervosa* in Tonga²²⁾.

Citrus greening disease and citrus tristeza virus (CTV) disease

1) Citrus groves are being devastated by the greening disease in the tropics

Citrus is most widely grown next to grape in the world. Citrus originated in southern China, India and Indochina peninsula. However, countries in Southeastern Asia are not included in the major citrus production countries, due to the epidemics of the greening disease in this area. Indonesia has experienced complete destruction of its citrus industry due to greening in northern Bali. The citrus-growing area of 12,000 ha in the early 1950s decreased to 4,000 ha in 1988 due to the disease in Thailand. In the Philippines, the area planted to mandarins and sweet oranges covered 25,000 ha in 1961–1962. By 1965 the area had been reduced to 17,300 ha and by 1974 to only 8,300 ha mainly due the greening disease^{1,18}.

Except for some islands located in Micronesia and New Caledonia, all of the countries and areas were found to be affected by this disease. The incidence of CTV was also commonly observed in all of the countries except for the islands in Micronesia, i.e. Saipan, Pohnpei and Palau. Development of the citrus industry in tropical and subtropical Asia, therefore, largely depends on the extent to which the control of greening disease is successful.

The causal agent of the greening disease is a fastidious bacterium, which is transmitted persistently by the Asian citrus psyllid, *Diaphorina citri*, while CTV is vectored non-persistently by aphids, mainly Toxoptera citricidus and Aphis gossypii¹³⁾.

2) Development of strains attacking pomelo

An important finding from the surveys was that pomelo, which was considered to be resistant to both greening and CTV diseases, has become susceptible recently in some areas. Greening was first noticed in the beginning of the 1950s in Taiwan by A. Sasaki^{5,20)}, but the incidence of this disease in Mainland China can be traced back to 1919 in northeastern Guangdong¹²⁾. Although pomelo began to be affected by greening disease in the beginning of the 1970s in Taiwan, in other countries it was yet free from this disease until the 1990s. Currently, greening disease is affecting pomelo in southern China and the Philippines, while pomelo grown in Vietnam and Thailand still seems to be free from this disease. It has been reported, however, that the infection of pomelo with symptoms of greening in Thailand was observed in a pomelo orchard in Chiang-Rai in 1994 (Bauman, personal communication, Koizumi, personal communication).

Surveys conducted in 1994 in some islands in the North Pacific revealed that greening did not occur in Saipan/North Marianas and Pohnpei, but that a pomelo-infective strain of greening was found in Palau/Belau which is located close to Mindanao, the Philippines. The islands in the South Pacific were considered to be free from greening, because of the absence of the vector insects. The survey teams, however, observed citrus trees with greening disease in Fiji, Western Samoa and Tonga. Since fruit trees normally reproduce vegetatively by grafting and layering, it is likely that all the scions and nursery stocks derived from an infected mother plant, which was introduced outside of the island, are congenitally infected with the greening pathogen.

In Japan, greening was first discovered by electron microscopy in Iriomote island, Yaeyama islands, Okinawa Prefecture by Dr. Miyagawa in 1989 when he was working for the FFTC Special Project. Since the incidence was limited to this particular island, an eradication program was successfully carried out. The FFTC organized a second survey to follow-up the status of greening in Okinawa in 1993. The survey team again found citrus trees affected by greening in this island. The causal agent was confirmed by DNA probes for greening prepared by Su and his colleagues at the National Taiwan University. In 1994, it was also detected in Okinawa island, suggesting that the distribution had expanded. Greening was found to be due to the strain which affects pomelo. Further inspection and eradication of the greening pathogen are recommended in order to prevent the spread of this disease to the other southern islands of Japan where the vector psyllids are common insects.

3) Control of greening disease and CTV

Greening is responsible for economic losses of entire citrus orchards within 5 or 6 years following an initial spread of epidemics²⁾. Eradication is an essential measure. Trees already affected by greening disease should be cut down and destroyed to ensure that they do not act as sources of inoculum.

So far, no plant resistance has been identified. Treatment with tetracycline reduces the population of bacteria, but symptoms usually reappear after 1 to 2 years. Heavy pruning and top-working of diseased trees and cutting off of infected branches for corrective control are often only useful for the elimination of potential inoculum of the greening.

Providing farmers with disease-free planting stock at a reasonable price is at the basis of any program to control virus disease in perennial crops. In Taiwan, since 1985, citrus planting stock, which is free from virus and greening, has been produced either by shoot-tip grafting with heat therapy or by nucellar line selection. There is also a budwood certification program, which ensures that the young seedlings distributed to farmers are free from diseases.

The feeding site of *D. citri* is confined to the flush or fresh shoots triggered mainly by rainfall in wet areas and irrigation in dry areas. Chemical control is applied at 10- to 20-day intervals in order to control psyllids which act as vectors during the sprouting period. Pesticides are commonly applied 36-48 times a year by Indonesian farmers¹⁷. Spray or trunk injection of systemic insecticides, such as

dimethoate and monocrotophos, has been suggested without any adverse effect on the fauna of natural enemies in the citrus grove¹⁸⁾. The use of IGRs (insect growth regulators) could be suggested for this purpose.

An ornamental plant often used as a hedge in Taiwan, jasmine orange, *Murraya paniculata*, does not harbor the pathogen of greening but is a favorite host plant of the psyllids. For this reason, jasmine orange should not be planted near citrus orchards. Biological control of the psyllids is carried out in Taiwan using an eulophid parasitoid, *Tamarixia radiata*, which was introduced from Reunion Island during the period 1984–1988. This parasitoid in association with an indigenous parasitoid, *Diaphorencyrtus diaphorinae*, is considered to contribute to the suppression of *D. citri*, particularly in jasmine orange³⁾.

When citrus orchards are to be established, the site of new orchards should be far enough from the old orchards to avoid or retard the infestation with psyllids. Shifting of planting areas from warm lowlands to cool highlands is also practiced to avoid the attack of psyllids in Taiwan. The CTV is spread by aphid vectors mainly by Toxoptera citricidus and to a lesser extent by T. aurantii and Aphis gossypii in a semi-persistent manner¹³⁾ and by propagation with infected buds. The control strategy of CTV disease is similar to that of other insect-borne virus diseases. The use of attenuated CTV for crossprotection has been applied for navel orange, Citrus sinensis, in Japan⁶⁾. The CTV is not readily transmitted between trees even in the presence of a fairly large number of vector aphids in a grove^{4,7)}. In fact, greening spreads more rapidly than CTV in newly planted orchards in Taiwan.

Table 3.	Difference in IPM strategy in relation to the occurrence o	f					
	insect-borne diseases						

	Area with diseases	Area without diseases		
Quarantine	Less important	Important to restrict the importation of infected plants		
Disease-free planting material	Should be developed by mass production system	Not necessary		
Cropping system	Annual planting or with the shortest vegetative growth	Perennial		
Protection measures from infection	Young plants must be protected from vectors ^{a)}	Control only when vectors may cause direct damage		
Objective	Good yield with healthy fruits for the first cycle of fruiting	Sustainable yield with high/standard quality of fruits for a number of years		

a): Because the earlier the infection with diseases, the heavier the damage.

Conclusion

Systems for the integrated control of virus diseases of fruit trees have 3 basic objectives: (1) the development of a production system for virus-free seedlings, (2) the protection of seedlings from the attack of viruliferous vectors, and (3) the elimination of virus sources. Various techniques are currently available for achieving these objectives and are applied in a number of countries in this region, partly as a result of the work of the FFTC survey team (Tables 2 and 3).

A very important problem identified by the members of the survey is the widespread incidence of fruit trees which showed multiple infections with different viruses or diseases. Many banana plants were infected with BBTV and CTV, while papaya was often simultaneously infected with PRSV and papaya mosaic virus (PMV), or with PRSV and PLDMV, and citrus with greening disease and CTV.

Simultaneous multiple infections of this kind often give rise to more severe symptoms than single infection. Presently, control is limited to the use of the integrated system mentioned above to prevent plants from being simultaneously affected by multiple virus diseases.

It was considered that the lack of techniques and expertise for precise indexing of the diseases for plant quarantine and production of disease-free planting materials was one of the major constraints on the implementation of IPM strategy for the insect-borne virus and virus-like diseases of fruit trees.

An IPM strategy has been suggested for the control of insect-borne diseases of tropical fruit trees as follows: (1) Distribution of disease-free planting material to growers. (2) Control of vectors; Planting time, use of barrier crops, silver mulch, netting, insecticides and biological control. (3) Shift of cropping system from perennial to annual crops. (4) Eradication of virus sources. Roguing and avoidance of cultivation of potential host plants close to orchards, e.g. cucurbits for PRSV and jasmine orange for greening. (5) Cross-protection techniques are not dependable or not available. The use of tolerant cultivars or antibiotics may often result in an increase of inoculum sources.

In conclusion, the difference in the IPM strategy between areas with and without disease is shown in Table 3. It should also be emphasized that some islands in the Pacific are now free from these devastating diseases, suggesting the importance of quarantine in preventing these diseases from invading the disease-free areas.

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