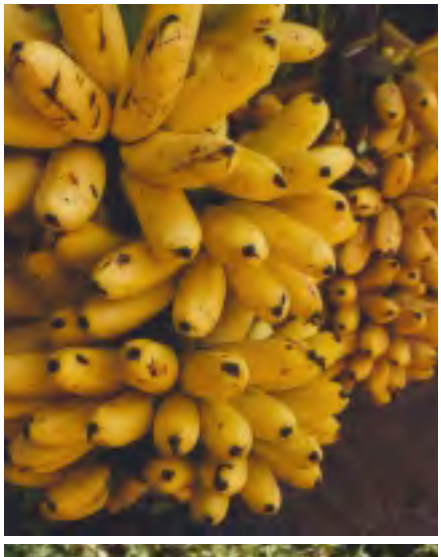
Managing banana and citrus diseases

Proceedings of a regional workshop on disease management of banana and citrus through the use of disease-free planting materials held in Davao City, Philippines - 14-16 October 1998

A. B. Molina, V. N. Roa, J. Bay-Petersen, A. T. Carpio, and J. E. A. Joven, editors



NTU

DOST-PCARRD

DA-BPI-DNCRDC

The mission of the **International Network for the Improvement of Banana and Plantain (INIBAP)** is to sustainably increase the productivity of banana and plantain grown on smallholdings for domestic consumption and for local and export markets. The Programme has four specific objectives:

- To organize and coordinate a global research effort on banana and plantain, aimed at the development, evaluation and dissemination of improved banana cultivars and at the conservation and use of *Musa* diversity.
- To promote and strengthen collaboration and partnerships in banana-related activities at the national, regional and global levels.
- To strengthen the ability of NARS to conduct research and development activities on bananas and plantains.
- To coordinate, facilitate and support the production, collection and exchange of information and documentation related to banana and plantain.

Since May 1994, INIBAP is a programme of the International Plant Genetic Resources Institute (IPGRI).

The **International Plant Genetic Resources Institute (IPGRI)** is an autonomous international scientific organization, supported by the Consultative Group on International Agricultural Research (CGIAR). IPGRI's mandate is to advocate the conservation and use of plant genetic resources for the benefit of present and future generations. IPGRI's headquarters is based in Rome, Italy, with offices in another 14 countries worldwide. It operates through three programmes: (1) the Plant Genetic Resources Programme, (2) the CGIAR Genetic Resources Support Programme, and (3) the International Network for the Improvement of Banana and Plantain (INIBAP).
The international status of IPGRI is conferred under an Establishment Agreement which has been signed and ratified by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mauritania, Morocco, Norway, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovakia, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine.

The **Food and Fertilizer Technology Center (FFTC)** is an international agricultural organization which has served small-scale farmers in the Asian and Pacific region since it was established in 1970. It does not carry out research, but collects and disseminates research results generated by other agricultural centers in the region. The aim is to provide farmers with improved practical information about new technology, so they can achieve higher yields and income.
The Center is based in Taipei, Taiwan ROC, and its staff are drawn from countries in the Asian and Pacific region. The Center is internationally funded, and works in close cooperation with other international and national agricultural agencies.

Citation: A. B. Molina, V. N. Roa, J. Bay-Petersen, A. T. Carpio, and J. E. A. Joven, editors. 2000. Managing banana and citrus diseases. Proceedings of a regional workshop on disease management of banana and citrus through the use of disease-free planting materials held in Davao City, Philippines, 14-16 October 1998. International Network for the Improvement of Banana and Plantain - Asia and the Pacific Network, Los Baños, Laguna, Philippines.

INIBAP ISBN: 971-91751-1-7

Cover Photo: Thai banana variety, Kluai Khai (upper left); young banana plants severely infected with BBTV (lower left); Philippine pummelo (upper and lower right).

IPGRI Headquarters
Via delle Sette
Chiese 142 00145
Rome, Italy

INIBAP
Parc Scientifique Agropolis 2
34397 Montpellier Cedex 5
France

INIBAP-ASPNET
c/o PCARRD
Los Baños, Laguna
Philippines

FFTC
P.O. Box 22-149
Taipei
Taiwan

Managing banana and citrus diseases

Proceedings of a regional workshop on disease management of banana and citrus through the use of disease-free planting materials held in Davao City, Philippines - 14-16 October 1998

A. B. Molina, V. N. Roa, J. Bay-Petersen, A. T. Carpio, and J. E. A. Joven, editors

NTU

DOST-PCARRD

DA-BPI-DNCRDC

The opinions in the publication are those of the authors and not necessarily those of INIBAP and FFTC.

Acknowledgments

INIBAP- Asia and Pacific Network (INIBAP-ASPNET) and the Food and Fertilizer Technology Center (FFTC) is grateful to all participants at the Regional Workshop on "Managing of Banana and Citrus through the Use of Disease-free Planting Materials" for their contribution to this proceedings.

INIBAP-ASPNET and FFTC would like to thank:

- Its local partners, the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD) and the Bureau of Plant Industry – Davao National Crop Research and Development Center (BPI-DNCRDC), Department of Agriculture for having provided staff support and local arrangements that ensured the workshop's success;

- Prof. H.J. Su of National Taiwan University (NTU) and Dr. S.C. Hwang of Taiwan Banana Research Institute (TBRI) who played a vital role during the planning and execution of this workshop;

- A.B. Molina, V. Roa, J. Bay-Petersen, A.T. Carpio, and J.E.A. Joven for their conscientious work as editors of the proceedings; and

- R.E.C. Mendoza who undertook the cover design and lay-out of the proceedings.

Table of Contents

Session III

Session IV

Annexes

Regional Workshop on Disease Management of Banana and Citrus:
The Use and Management of Disease-Free Planting Materials

October 14-16, 1998
Insular Century Hotel
Davao City, Philippines

Opening

Mr. Nerius I. Roperos
Director
Bureau of Plant Industry
Philippines

Dr. Iwao Watanabe, deputy director of FFTC, Dr. Agustin Molina, regional coordinator of INIBAP-ASPNET, Dr. Ramon Valmayor, colleagues in government and private sector, distinguished guests, ladies and gentlemen, good morning.

I am deeply honored to join you today in this very significant technical undertaking – the Regional Workshop on Disease Management of Banana and Citrus: The Use and Management of Disease-free Planting Materials. This workshop brings together experts and leading personalities in the banana and citrus industries with the aim of improving the banana production and reviving the once flourishing citrus industry.

We are now faced with the problem of meeting the demands of farmers and growers for sufficient supply of high quality planting materials. The need is more acute in local bananas where viral diseases, specifically banana bunchy top, a very serious problem in smallhold farms. The disease is quite widespread all over the country and there's still lack of well-coordinated control strategies among growers, extension workers, and local communities. The lack of disease-free materials in adequate and affordable quantities has aggravated the crop protection problem and stunted the development of the local banana industry.

Likewise, the citrus industry has been declining in the last 15-20 years because of the greening problem as well as citrus canker and severe strains of tristeza.

In most cases, these problems can be solved in the field through conventional control measures such as cultural practices, field sanitation, chemotherapy, etc. An exception to this rule is the problem coming from graft trasmissible virus and virus-like diseases for which the only known cure is prevention by propagating virus-free planting materials. This is usually within the framework of a sanitation program based on diagnostic detection and elimination of the causal agents and the maintenance and distribution of healthy stocks.

In behalf of the Department of Agriculture through the Bureau of Plant Industry, I am extending a very warm welcome to all of you and may your stay in Davao bring new friendship, cooperation, and pleasant memories.

And finally, may I also extend our heartfelt gratitude to the valuable support provided by FFTC, TBRI, INIBAP, and PCARRD to this workshop.

Good Morning and Mabuhay!

Dr. Iwao Watanabe
Deputy Director
Food and Fertilizer Technology Center for the Asian
and Pacific Region
Taiwan

Distinguished speakers and guests, ladies and gentlemen, it is a great privilege for me to be with you here on this beautiful island of Mindanao. I would like to welcome you all to this meeting and hope you will find it a rewarding and interesting one. I would like to express special thanks to Dr. William D. Dar, Secretary of Agriculture, who has honored us with his presence today. I would like to welcome the present and past Regional Coordinators of INIBAP, Dr. Molina and Dr. Valmayor. Both are experienced scientists and administrators, who have been working hard to promote the banana production in the Philippines and other countries in the region. This is the first joint activity between the Food and Fertilizer Technology Center and International Network for the Improvement of Banana and Plantain. I hope it will be the forerunner of many years of successful cooperation between our two organizations.

I would also like to thank the officers and staff of the Davao National Crop Research and Development Center of the Bureau of Plant Industry. A meeting of this kind takes many months of preparation. Their hard work and efficiency have resulted in these excellent arrangements we are enjoying now. Thank you very much.

The meeting today is a very important one. Citrus and banana have become major cash crops for farmers in this region. However, orchards and plantations are being ravaged by diseases. In the case of viral and greening diseases, these diseases cannot be treated once the plant has become infected. The only remedy is to remove the diseased trees and replant. However, infection is so widespread that many mother trees and nursery stock are also infected. Many of you may have encountered the tragedy of farmers whose orchards were ruined by diseases and replant at great expense, only to see the same catastrophe happening all over again. I hope that the information from this meeting will help avoid such tragedies in the future. The FFTC will do its best to disseminate these information to those who may need it.

Since 1991, the FFTC has been carrying out a special project funded by the Japanese Government on viral diseases of fruit trees. In the early years, work focused on surveys to see what viral diseases were prevalent, and methods to diagnose and index viral pathogens. As you all know, viral diseases in the early stages are often without any visible symptoms, and are impossible to detect with the naked eye. Using new diagnostic techniques,

the FFTC survey teams found that many viral diseases of fruit trees were much more widespread than was previously thought, and that many trees were infected by several different viruses at the same time. Many others suffered from mild strains of virus which produced no visible symptoms but reduced fruit yields.

At present, the FFTC is helping to promote disease-free nursery systems, so that fruit producers in Davao and elsewhere will be able to buy certified disease-free seedlings. This is the only way diseases can be controlled. Since disease-free seedlings are quite vulnerable to infection, this work must also involve ways to keep plants free from disease after they are planted in the orchard or plantation.

Those of you present in this meeting are in the forefront of the battle to control viral diseases of fruit trees. I hope the information presented in this meeting will help you in your future efforts. Aside from discussing the technology of producing disease-free seedlings, I hope you will also consider efficient systems of producing affordable seedlings for small-scale farmers. In Taiwan and other countries, there have been some successes in treating banana as an annual crop and replanting every year with banana seedlings which are mass produced by tissue culture. This practice needs a well-developed system of production and distribution to ensure that all banana growers have enough seedlings when they need them.

Thank you again for your presence in this meeting. To the foreign participants, my thanks again for making the long journey to be with us here today. I hope you all have a pleasant stay in this hospitable and charming country. I hope too that you will return home with new ideas about how fruit farmers in your country may keep their orchards disease-free, so that they will enjoy a reasonable income from all their hard work.

Dr. William D. Dar
Acting Secretary
Department of Agriculture
Philippines

Dr. Iwao Watanabe, Dr. Agustin Molina, friends and colleagues, ladies and gentlemen:

We take pride in being part of this regional undertaking. Nothing can be more timely than this workshop in putting to task a regional effort toward a more concerted action that is meant to give banana and citrus the attention these crops deserve.

This Workshop is well placed and well timed. You have chosen a very appropriate location, Davao being the country's premier banana area. Nothing can truly be more timely and relevant than the holding of this workshop. President Joseph Estrada has now focused his vision to modernize Philippine agriculture by empowering the smallhold agriculture and fisheries sector. The President's vision clearly paints a future that promises every Filipino better and more food.

We, at the Department of Agriculture, feel committed and are resolute in making this vision take shape. This has given the Department renewed vigor – a challenge you may want to call – to advance the country's global competitiveness through an empowered people to utilize to the full all resources at their disposal, which the government complements with matching mechanisms that will spur their participation and inherent decision-making capability. This is the foundation of a future framed by President Estrada's vision. This is the future we envision for the small man. This is a dynamic future we see. This dynamism is now ensconced in the Agriculture Department's guiding vision and philosophy which is comprehensively packaged in the recently completed Framework for Agriculture and Fisheries. This document not only guides us toward that vision, but also provides us the impetus to work hard in enhancing the country's agricultural and fishery development.

Past experiences and lessons learned made us discern what really works and what does not, distinguish the fundamentals from peripheral constraints, and take cognizant of the rightful roles of government and private sectors. This insightful exercise at knowing where we are, what we are now, how do we see the future of agriculture from today, and how it is poised to compete in the global village has been our guiding principle that we hope shall provide us the conscience to pursue our ten-point modernization agenda. In summary, these are:

---

* Keynote Address read by Dir. Nerius I. Roperos in behalf of Dr. William D. Dar, Acting Secretary, Department of Agriculture.

1. Expansion and revitalization of productivity programs;
2. Quick-response / intervention to calamity and disaster situations;
3. Irrigation;
4. Farm-to-market roads and related infrastructure;
5. Agriculture and fisheries research and development;
6. Extension, education, and training;
7. Rural finance;
8. Food price stabilization;
9. Private sector participation and empowerment of farmers and fisherfolk;
10. Renewal of DA (Department of Agriculture) bureauracy

Our government cannot afford to concentrate solely on the development of high-yielding banana and citrus varieties that promise us better yield, but must think of implementable systems that will truly alleviate the plight of poor farmers against pests and diseases of these important crops, if our development goal is to help them increase production, provide them better income opportunities and gain competitive edge in global market.

Banana and citrus have become two of our priority fruit commodities in line with the country's food security and poverty alleviation programs, which are directed at increasing income, employment opportunities and nutritional levels through high value / commercial and nutritional crops.

The thrust of the Philippine government now is to rehabilitate the citrus industry, with assistance from the French and Israeli governments. Efforts have been directed through this foreign assistance to beef up production of disease-free citrus planting materials. Our concentration has also been focused at highly competitive citrus varieties, such as calamansi and pomelo.

You are certainly aware of the position of the Philippine banana industry in the international market. The country has maintained competitiveness of our banana exports and has developed, through the years, great strides to sustain the program on banana that is beneficial to both big and small farmers.

The government is very much focused at improving and developing our local banana varieties. This will allay fears that the local varieties are neglected. The Department of Agriculture carries the same passion that small farmers feel that is to sustain disease management programs that will see no more destruction of banana plantings. This has been so when fighting off such deadly scourge of banana bunchy top disease.

We are likewise relentless in developing and implementing postharvest and marketing practices that will make farmers and consumers enjoy fruit harvests. This is by improving refrigeration systems that will equally improve and, at the same time, prevent losses during transport of banana fruits from farm to market.

Our concentration now is to develop a cold chain system that will reduce losses incurred from shipping such as perishable good as banana fruit. We hope to see no more shipments of, for example, our lakatan variety from Mindanao to Manila without cold storage that naturally makes producers lose about 30% of the product because of conventional transport, a price that consumers have to pay when producers jack up the price to make up for the 30% loss.

In your hands now is the enormous responsibility to help the region build a strong banana and citrus program. You have in your midst the task

and the challenge to show that disease management of these essential crops will seem more essential if greater understanding is made such that safeguarding the industry through systematic disease management is far more important than undertaking disease control. Conversely, it should be understood that the benefits from such efforts can prove unattainable if nothing is done to enforce systematic use of management system.

I hope you will dwell upon these issues and develop strategies that will give workers the responsibility and the authority to carry on an improved disease management programs for banana and citrus.

It is in endeavors such as this Workshop that trends and development in science and technology are monitored. I wish that you would develop shared or common system that would benefit our region's banana and citrus industries. There are needed standards that require monitoring to enable us to maintain such standards for global sharing of banana and citrus disease management technology.

On the part of the government, specifically the Department of Agriculture, we shall always put as high priority your recommendations that we further hope will provide directions and new insights that we need now, more than ever, in pursuing greater sustainability of our food security and poverty alleviation program.

I am an unabashed optimist. I believe that there always comes something novel in deliberations of this nature, having been into several of such activities since and before. I shall wait for the results of your deliberations and proceedings with interest.

With the Estrada Administration just past its first 100 days, none can be more welcome than the compliments of your coming to participate in this August gathering with the launching of *Maka*MASA, auspiciously the final day of this workshop on 16 October, which, very timely indeed is the day we are celebrating twin occasions – the Anniversary of the Department of Agriculture and the World Food Day.

*Maka*MASA is my Department's revitalized formula for embodying the spirit of Agriculture and Fisheries Modernization Act of 1997, which now serves as a blueprint for the government's modernization efforts. As a medium-term agricultural development program, *Maka*MASA, which in Filipino means *Makapagpapabagong Programa Tungo sa Masagana at Maunlad na Agrikultura at Pangingisda,* is geared to expand and put more life to previous productivity programs.

As I have emphasized in the beginning, you have chosen no other more appropriate venue for this Workshop than Davao, the fabled land of smiles, of southern exotica that, I am afraid, will just be as irresistible to ignore as to get its effects linger on even when you are already quite settled back home. Do not ever leave the city without getting a taste of its exquisitely different variety of the king of all fruits, the tempting durian. Davao not only prides itself with its world-class banana, but also with its citrus, especially its suha, the valued pomelo.

This Queen City of the South beckons. Its wide expanse of pristine beaches and a serene blend of rural-urban life will make you want to excuse from your hectic schedule and delight yourself with the city's fascinating charm.

Thank you and I wish you all healthy exchange of ideas and please make your Philippine visit pleasant and truly exotic.

Good Day and MABUHAY!

Dr. Agustin B. Molina
Regional Coordinator
INIBAP-ASPNET

Mr. Nerius Roperos, Director of the Bureau of Plant Industry, Department of Agriculture, Dr. Iwao Watanabe, Deputy Director of FFTC, banana and citrus experts from various countries as well as from the Philippines, colleagues from the industry, other officials from PCARRD, FFTC and BPI, ladies and gentlemen.

In behalf of INIBAP-Asia and the Pacific Network, I would like to express my thanks to all of you for coming together to address very important concerns which are seriously threatening two important fruit crops in the region. In the case of banana, viruses present a significant constraint to the productivity and even to the survival of the banana industry. The damaging effects of banana viruses such as the Banana Bunchy Top Virus (BBTV) and the recently reported epidemic of Banana Bract Mosaic Virus (BBrMV), are most felt by smallholding banana growers who do not have the expertise and economic resources to implement an effective disease management program. In the Philippines for instance, the smallholding subsistent Lakatan industry have practically disappeared due to severe epidemic of BBTV. BBrMV has been reported to be attacking the important cooking banana "Saba." Epidemiologically, banana viruses and some major diseases of citrus, are spread and transmitted through planting materials such as infected suckers in the case of bananas. To manage these diseases, it is obviously important to use disease-free planting materials as a part of an effective Integrated Disease Management program. Unfortunately, latent infection of these diseases, particularly on suckers as well as tissue culture plantlets derived from symptomless, infected mother plants are difficult to diagnose. Fortunately, some R & D advances on detection and its application in IPM has been successfully tried in countries such as Taiwan and Australia. Similar efforts are also in place in other Asian countries.

This workshop is very relevant as banana and citrus experts from various countries come together to address the topic of management of the major banana and citrus diseases through the use of disease-free planting materials. I also expect that we will be sharing our expertise and experiences on the dynamics and nature of these diseases so that we can put together other tactics that would compliment clean planting materials in the management of these diseases. We also hope that we can identify areas of R & D to further our efforts in the management of these damaging diseases.

I reiterate our thanks to all of you, especially those who come from other countries, in finding time to participate in this Workshop. I also thank our partners – FFTC, PCARRD and BPI, in joining with INIBAP in making this important workshop a reality. I hope that in the next few days, we will have a productive meeting.

Session I

Epidemiological Review on Citrus Greening and Viral Diseases of Citrus and Banana with Special Reference to Disease-free Nursery System

Hong-Ji Su
Department of Plant Pathology
National Taiwan University

Abstract

Virus and virus-like diseases of citrus and banana have been causing considerable damage to fruit production in tropical areas. This is due to their systemic invasion and common spread through infected budwoods, vegetatively propagated seedlings, and transmission by insect vectors. The heat-tolerant form of greening locally known as Huanlungpin in Taiwan, has seriously affected citrus trees in the tropical and subtropical regions of Asia. This caused great damage to the citrus industry by shortening tree lifespan. Most citrus cultivars, except pummelo, were already susceptible to the Asian form of greening organisms (GO) before the 1970s. However, pummelo become infected by a new GO strain in Taiwan and Southeast Asia in 1971. Citrus tristeza virus (CTV) is the most common and destructive virus in the western hemisphere, and has a worldwide distribution. Various new strains of CTV have recently evolved and invaded pummelo, sweet orange, and mandarin cultivars resistant to the old strains of CTV. The bunchy top disease is a common and destructive viral disease of banana in the Asian and Pacific regions. The banana mosaic, caused by CMV, has become a major threat after the widespread use of tissue cultured plantlets. Banana streak badnavirus and bract mosaic virus have spread to many banana areas in the Pacific regions in recent years.

The virus and virus-like diseases have become one of the serious constraints for the production of tropical fruits in the Asian and Pacific regions. The epidemiological data of the aforementioned virus and virus-like diseases, which are indispensable for formulating integrated control measures, are reviewed in this paper. These vector-borne systemic diseases have been effectively controlled by integrated control measures including production and cultivation of virus-free seedlings, elimination of inoculum sources, and prevention of reinfection through IPM of vector insects. Establishment of pathogen-free nursery system is primarily important to prevent prevalence of the diseases. The precise and rapid indexing techniques are indispensable for management of virus-free nursery system. The development and application of molecular diagnostic probes for detection and indexing of virus and greening pathogens are primarily important in the production of pathogen-free seedlings.

Introduction

Banana and citrus are the most important fruit crops in Taiwan. These are produced for local consumption and export to other Asian countries. Some systemic virus and virus-like diseases have been causing considerable damage to fruit yield and quality, and have become one of the serious constraints in the production of tropical fruits in the Asian and Pacific regions. The bunchy top disease has been the most common and destructive viral disease of banana since the beginning of this century in the Asian and Pacific regions. Several outbreaks have become the limiting factor for the growth of the banana industry in Taiwan during the past decades. Banana mosaic caused by cucumber mosaic virus (CMV) was first reported in New South Wales in 1929 and in Central America in 1957. This disease became prominent in Taiwan in 1974, and severe outbreaks occurred recently after the widespread cultivation of tissue-cultured plantlets. Citrus greening was first reported in South Africa in 1947. The psyllid borne virus-like disease caused by fastidious bacteria has been devastating the citrus industry in Taiwan since 1951. This disease is prevalent in tropical and subtropical citrus areas in Asia and South Africa. Citrus tristeza virus (CTV) was assumed to have originated from China. The CTV caused great damage to the citrus industry in South America and Africa in the 1920s, and has a worldwide distribution. A new strain, so-called CTV-D, has appeared and caused serious dwarfing of pummelo in Taiwan in 1981. The virus was initially assumed to be a non-threat in the tropics, however, some severe CTV strains have evolved in Southeast Asia in recent years and have been causing severe stem-pitting and stunting which affect fruit yield and quality. These diseases are transmitted by vegetative propagation of seedlings such as suckers, cuttings, and bud scions for grafting as well as insect transmission. These diseases are generally controlled by integrated control measures including cultivation of pathogen-free seedlings, elimination of inoculum sources, and prevention of secondary spread by vector insects. Establishment of pathogen-free nursery system is primarily important for preventing prevalence of these diseases. Shoot-tip micrografting of citrus and tissue culture combined with heat therapy have been utilized for obtaining virus-free foundation stock of citrus and banana cultivars in Taiwan. The precise and rapid indexing techniques are indispensable for management of pathogen-free nursery system. Development and application of molecular diagnostic probes including monoclonal antibodies, DNA probes, and primer pairs were for formulating indexing techniques. The virus-free nursery system for controlling virus and virus-like diseases of citrus and banana has been operationalized through micrografting, tissue culture, and indexing in Taiwan.

Epidemiological Review

Citrus greening, a virus-like disease of citrus locally known as Likubin or Huanglungpin in Taiwan, has seriously affected citrus trees of all cultivars and caused great damage to citrus industry by shortening tree lifespan since the 1950s. The disease was first reported from South Africa in 1947, although the disease has been known since 1929. Likubin was reported in Taiwan in 1951, while a similar disease known as Huanglungpin (yellow shoot) was reported in Mainland China in 1943. The virus-like disease known as leaf

mottle yellows in the Philippines, citrus dieback in India, and citrus vein phloem degeneration in Indonesia, has been devastating all citrus-growing areas in Asia and South Africa.

The fastidious bacteria (GFB) is nonculturable and submicroscopic walled prokaryote existing in sieve tube. Electron microscopy, using serial sections and stereomicrographing confirmed the presence of mature forms of the pathogen, generally rigid rods measuring 350-550 x 600-1500 nm, surrounded by a two-layered envelope, 20-25 nm thick. However, GFB bodies are pleomorphic during growth. The Asian greening organism produces symptoms in either warm (27-32°C) or cool climate, and is classified as heat-tolerant. The South Africa greening organism belongs to a heat sensitive form inducing severe symptoms at cool temperatures (22-24°C). Most citrus cultivars except pummelo were susceptible to the Asian form of GDB. However, pummelo had become infected by a newly evolved strain of the pathogen in Taiwan since 1970. Pummelo trees grown in the Philippines, East Malaysia, Southern China, and Palau have been found to be infected with GFB in recent years. The mandarin isolates of Asian GFB induced severe greening symptoms in mandarin and sweet orange trees but mild symptoms were developed in pummelo. The pummelo isolate caused severe symptoms in pummelo, mandarin, and sweet orange. It is assumed that Asian GFB might include such strains as mandarin strain and pummelo strain. The Thailand pummelo, formerly free of greening, recently became infected presumably owing to the occurrence of the new pummelo strain.

No commercial citrus cultivars in Taiwan was found to be resistant to greening. Although the disease syndrome differs to some extent depending on citrus varieties. Common symptoms are yellowing of the veins and adjacent tissues, followed by yellowing or mottling of the entire leaf. This is followed by premature defoliation, dieback of twigs, decay of feeder rootlets and lateral roots, decline in vigor, and ultimately the death of the entire plant. Trees affected with greening become stunted, bear multiple off-season flowers, most of which fall off, and produce small off-shaped fruits with thick and pale green peel.

Host range and epidemiology. The Asian form of greening has been rapidly spread by Asian psylla (*Diaphorina citri*), while African form of greening is transmitted by African psyllid (*Trioza erytreae*) in persistent manner. Biotypes of Taiwan psyllids are not a highly efficient vectors of greening. Epidemics occur only when high vector populations and extensive inoculum reservoir are present. Natural spreading occurs during sprouting time when vector population is high. Transmission of greening via infected budwood plays an important role in disease spread.

The greening agent can infect most citrus species, cultivars, and hybrids. Most mandarins, sweet oranges, and mandarin hybrids are severely affected. Pummelo became liable to Asian greening in recent years. The varieties of rootstocks do not affect the disease resistance of scion varieties. Multiplication of GFB was detected in graft-inoculated woody apple (*Lemonia acidissima*), and Chinese box orange (*Severinia buxifolia*) suitable host of vector psylla. No GFB growth was detected in Jasmine orange (*M. paniculata*) and curly leaf (*Murraya euchrestifolia*). The Chinese box orange, a wild shrub common in Taiwan, Southern China, and Southeast Asia, harbors the GFB persistently, and might be an alternative host of the pathogen.

Indexing. Greening is commonly identified in the field by foliage and fruit symptoms. Further diagnosis requires indexing on susceptible citrus seedling by graft inoculation. Because of low population and uneven distribution of GFB, bioassay with indicator plant is a time-consuming and uncertain method. DNA probes and Polymerase Chain Reaction (PCR) with adequate primer pairs were developed through DNA cloning and sequencing of GFB. Dot hybridization (DH) with the selected DNA probe showed specific and constant sensitive reaction with DNA extract from greening-afflicted citrus plants. DH with DNA probe has been commonly applied for the detection and ecological study of the pathogen. Detection of GFB in symptomless citrus plants and alternative host plants could be accomplished by DGH test, which has been commonly applied to indexing citrus foundation stocks and pathogen-free seedlings. The sensitivity of GFB detection is uplifted by the development and application of PCR analysis with adequate primer pairs recently (Hung *et. al*, unpublished).

Citrus Tristeza

Citrus tristeza closterovirus (CTV) is assumed to have originated in China long time ago. Tristeza known as quick decline in the United States is the most destructive disease of citrus in the western hemisphere and distributed worldwide. Less than two decades after tristeza was introduced from Africa into South America in the 1920s, the disease wiped out the citrus industries of Argentina, Brazil, and Uruguay. The virus caused the Hasaku dwarf seriously in Japan. Failure of sweet orange, or grapefruit budded onto sour orange stock, is the distinct damage of CTV. The virus latently infects tolerant citrus cultivars. In recent decades, some new virulent strains of CTV have been developed through frequent evolution worldwide. The extremely destructive forms of CTV pose serious threats to citrus industries worldwide. CTV-D, a new strain, has appeared and caused dwarfing of pummelo in Taiwan since 1981. Pummelo was formerly resistant to CTV. In 1988, a severe new strain, Capao Monito appeared in Brazil and caused serious damage to sweet orange. Some new stem-pitting strains attacking navel orange in Peru and Australia, grapefruit in South Africa and Australia, Valencia sweet orange in Indonesia and China, and mandarin and calamondin in Thailand, Philippines and East Malaysia were also found in recent years.

The disease is caused by citrus tristeza closterovirus with flexous rod-shaped particles approximately 12 x 2,000 nm. The CTV is present worldwide and consists of a complex of virus strains. Its strain variation is very complicated and ranges from mild isolates to severe and destructive stem-pitting isolates.

Host range and epidemiology. CTV infects nearly all species, cultivars, and hybrids of citrus. The only known nonrutaceous host is Passiflora. The virus is transmitted by vector aphids in semipersistent manner, and via infected budwoods. The efficiency of transmission varies with the aphid species, virus isolates, and the donor and receptor hosts. *Toxoptera citricida* is the most efficient vector while *Aphis gossypii* is an efficient vector of some isolates. *A. citricola* is a less efficient vector. The virus causes different symptoms on citrus plants depending on the virus strain, the variety of citrus, and the scion-rootstock combination. Sweet orange, or grapefruit budded on sour orange rootstock is particularly liable to CTV by producing severe

decline symptoms including dwarfing, vein clearing, and fruit atrophy and deformation. The mandarin cultivars are generally tolerant to the virus. Pummelo cultivars are hypersensitively resistant to the virus except pummelo stempitting strain (CTV-D).

Indexing. Mexicum lime, also known as key lime, is the best indicator for indexing CTV. Leaves of the lime develop different symptoms of vein clearing and stem pitting with different virus strains. Eureka lemon, sour orange, and grapefruit are used for indexing seedling yellow strain by producing yellowing and atrophy symptoms on upper younger leaves. Such different cultivars as sweet orange, pummelo, and mandarin are used for identifying stem pitting strains of CTV. The CTV is detected effectively by ELISA with polyclone and monoclonal antibodies. Development and application of monoclonal antibodies through hybridoma technology are utilized for the differentiation of certain strains (CTV).

Banana

Banana Bunchy Top

Banana bunchy top virus (BBTV) disease has been the most common and destructive viral disease of banana since the beginning of this century in Taiwan and other banana-growing areas in the Asian and Pacific regions. BBTV was first recorded in Fiji in 1891 and in Taiwan in 1892. Serious epidemics of BBTV occurred in Australia between 1913 and 1926. These led to the establishment of legal control measures to control the disease in Australia. Several occurrence of outbreak became the limiting factor for banana industry in Taiwan during the 1900s, 1960s and 1980s. A serious epidemic has been reported recently from Pakistan. The virus causes substantial disease outbreaks if the aphid vector, *Pentalonia nigronervosa* is present. Several countries such as Australia, Taiwan, and Philippines have implemented extensive elimination program for controlling epidemics. The disease has spread through the South Pacific (Guam, Hawaii, Western Samoa, Tonga, Fiji, etc.), Asia (China, India, Indonesia, Malaysia), New Caledonia, (Okinawa) Japan, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand and Vietnam) and Africa (Burundi, Congo, Egypt, Gabon, Rwanda, and Zaire). Amazingly, the disease has not reached Central and South America.

Banana bunchy top virus (BBTV) has been consistently associated with the disease. The virus has 20 nm isometric virions with a coat protein of 20.1 kDa and a multicomponent single-stranded DNA (1.1 kb) genome. The symptom expression of infected Cavendish banana plants is influenced by the strains of BBTV (i.e. distinct symptoms of bunched, atrophy leaves induced by severe strain of the virus; slightly stunting and vein clearing symptoms caused by intermediate strain; and mild/latent strains induces symptomless appearance). The different strains of BBTV are differentiated by PCR (polymerase chain reaction) with three primer pairs.

Host range and epidemiology. The virus is transmitted vegetatively, through suckers and tissue culture, and by the aphid vector *Pentalonia nigronervosa* in semi-persistent manner. No mechanical transmission has been reported. *Musa* species and cultivars are the main hosts. There are evidences which

point to the existence of alternative hosts i.e. garland flower (*Hedychium coronarium*) and canna (*Canna indica*). Meristem-tip culture combined with heat therapy (35°C, three months) is effective in obtaining virus-free plantlets of Cavendish banana. The virus causes substantial epidemic if the aphid vector is present.

Indexing. The virus can be detected by ELISA (enzyme-linked immunosorbent assay) with monoclonal antibodies. Adequate buffer solution have to be selected for virus extraction in ELISA test in order to eliminate interfering substances in banana latex and stabilize virus extract. The adequate buffer for extracting BBTV is 0.5M Tris buffer (pH 7.5) containing 0.1% Na-DIECA, 5% sucrose and 0.5% skim milk. PCR assay with adequate primer pairs showed high sensitivity and specificity in detecting and differentiating BBTV strains.

Banana Mosaic (Infectious Chlorosis)

The different strains of cucumber mosaic cucumovirus (CMV) cause banana mosaic, infectious chlorosis, and heart rot disease. These diseases are present in most banana growing areas of the world. Banana mosaic caused by CMV was first reported in New South Wales in 1929 and in Central America in 1957. The disease became prominent in Taiwan in 1974. Occasionally, severe outbreaks occur in Taiwan after plantlets derived from tissue culture (TC) are widely cultivated. The causal virus (CMV) has a very wide host range that serve as inoculum sources causing high frequency of infection. Numerous strains exist, varying from those inducing severe or mild symptoms to those not causing symptoms. The heart rot strain and severe leaf distortion strain found in Morocco and Taiwan are particularly destructive.

The CMV with a tripartite single stranded RNA is packaged in icosahedral particles about 28 nm in diameter. Satellite RNA, CARNA 5 has been associated with many strains of CMV, but has not been found in those strains infecting banana. Three strains of CMV were identified to cause banana mosaic disease of different severity. The severe strain of solanaceous strain group causes severe mosaic with distinct leaf distortion; mild chlorotic streak and finite mottle are caused by mild strain of solanaceous CMV; while legume strain systemic causes severe mosaic with necrosis or heart rot of spindle leaves.

Host range and epidemiology. The causal virus, CMV, infects extremely wide range of host plants including numerous dicotyledon and monocotyledon species. The virus is transmitted by more than 60 aphid species in nonpersistent manner. In banana, *Aphis gossypii*, *Myzus persicae*, *Rhopalosiphum maidis* and *R. prunifoliae* are the important vector aphids. Bananas are not the normal hosts for the aphids. Cotton aphid, *A. gossypii* having wide host range, is the most important vector aphid. Infected crop plants and weeds of solanaceous and legume species are the usual sources of inoculum for infecting bananas. TC-banana plantlets are more liable to CMV than suckers as planting seedlings. The banana plants over 1 m high, become tolerant to CMV infection. Disease incidence has been much greater in Cavendish cultivars than in Gros Michel.

Indexing. ELISA test with monoclonal antibodies was developed for indexing CMV in banana for the management of virus-free nursery system in Taiwan. The virus can be reliably detected by ELISA using polyclonal and monoclonal antibodies against CMV. The adequate buffer for extracting CMV from banana 0.2 M potassium phosphate buffer (pH 7.4) containing 0.% sodium sulfite. The virus can be identified by mechanical inoculation to indicator plants, e.g. *Chenopodium amaranticolor, C. quinoa*, and cowpea (*Vigna unguiculata*).

Banana Streak

Banana streak disease was first reported in the Ivory Coast in 1974. The disease also occurred in Morocco, Jordan, Rwanda, Mauritius, Tanzania and Zanzibar. Leaf-stripping symptoms in Mysore (AAB) in Trinidad, previously considered to be a physiological disorder, have recently been found associated with banana streak badnavirus (BSV) infection. There is the potential for serious yield losses with some virus isolates. Disease incidence varies between countries and this may be related to different virus strain, banana cultivars and vector activity. Virus infection was recently found in exotic Mysore banana in Taiwan. Tissue-cultured plantlets of Cavendish banana are very susceptible to the virus. Severe streak symptoms with necrosis were developed on the plantlets of Cavendish banana (AAA) inoculated by citrus mealybug. The disease has recently been found in most banana producing countries in the Central and South Amercia, Southeast Asia, South Pacific, Australia, and Africa.

The disease is caused by BSV consisting of nondeveloped bacilliform particle measuring 120-150 x 30 nm. Particles contain a circular double-stranded DNA genome, 7.4 kb in size. Three serologically and genomically distinct isolates of BSV have already been identified. The isolates of sugarcane bacilliform badnavirus also produced chlorotic streak symptoms in inoculated Dwarf Cavendish. Sugarcane bacilliform badnavirus was found in sugarcane cultivars in Taiwan in 1995.

Host range and epidemiology. Natural hosts include *Musa* species and cultivars, and sugarcane cultivars. Symptoms vary with virus isolates and banana cultivars. Most isolates produce broken or continuous chlorotic streak or fine spindle-shaped spots, which are first chlorotic, then become black streaking in older leaves. Some isolates produce severe necrosis causing heart rot of spindle leaves. Some isolates produce fine indistinct broken streaks. Bunches may be reduced in size. Symptomless infection occurs frequently. Symptoms are often confused with those of CMV. BSV is transmitted in a semipersistent manner by citrus mealybug, *Planococcus citri*. The virus is principally transmitted via suckers and tissue-cultured plantlets derived from infected sources. Symptomless carriers may serve as important sources of disease spreading. It is possible that infected sugarcane may be a source of virus for banana infection.

Indexing. Serological detection of BSV is complicated by the occurrence of a wide degree of serological diversity among viral isolates. An antiserum raised against many isolates is capable of detecting all known isolates by ISEM in partially purified extract. The PCR analysis with primer pairs (F1: CAA CTC AAG AGC CTA GRA TGC; R2: TAC CTC CGA CCG TAT TTC CAG) (J. Thomas) was found to be a reliable method of detecting the

Taiwan isolate of BSV in AAB (Mysore and Latundan) and AAA (Cavendish) bananas (Su, unpublished).

Disease Management

Integrated control of citrus virus and greening diseases

The systemic virus and greening diseases are transmitted by vegetative propagation as well as insect transmission. These diseases may readily prevail if the mother stocks and seedlings are infected latently with these pathogens. Establishment and performance of pathogen-free nursery system is primarily important to prevent prevalence of the diseases. Shoot-tip micrografting combined with heat treatment will be the most promising method to make infected plant virus free. The precise and rapid indexing techniques are indispensable for management of pathogen-free nursery system. In order to combat these systemic diseases, the following integrated control measures have been adopted in Taiwan:

- Performance of pathogen-free foundation stocks through a modified method of shoot-tip grafting, heat therapy or nucellar line selection (except pummelo), and pathogen-free nursery system;
- Cultivation of healthy citrus seedlings under a budwood certificate program with indexing;
- Prompt elimination of infected trees and alternative host plants as inoculum sources in order to prevent reinfestation;
- Protection of healthy citrus seedlings grown in field from reinfestation through the following:

    a. Spraying of insecticide at 10-20 day intervals during critical infection periods for controlling vector psyllids and aphids, and releasing eulophid wasp, *Tamarixia radiata,* for biocontrol of psyllids.
    b. Preimmunization of health foundation stocks or mother trees with protective mild strains of CTV as cross protection against severe strains of CTV.
    c. Disinfection of CTLV – or exocortis viroid-contaminated pruning tools by dipping in 1% NaOCL (bleach) and rinsing with 5% acetic acid (vinegar) + 2% mineral oil.

Integrated control of banana virus diseases

These viral diseases are transmitted by vegetative propagation such as suckers and tissue-cultured (TC) seedlings as well as insect transmission. The systemic viral diseases of banana are generally controlled by the integrated measures including (1) planting of virus-free seedlings and (2) prevention of reinfestation by elimination of inoculum sources and vector control.

- **Production and cultivation of virus-free seedlings.** These diseases might readily prevail if the mother stocks are infected latently with the causal viruses. Establishment of virus-free foundation is primarily important for producing healthy plantlets through tissue culture. The virus-free

TC-seedlings of banana are widely grown for controlling banana viruses in Taiwan. The disease-free nursery system is well maintained by using precise and rapid indexing techniques including ELISA and PCR tests mentioned above. Healthy suckers from non-infected mother stocks are also planted.

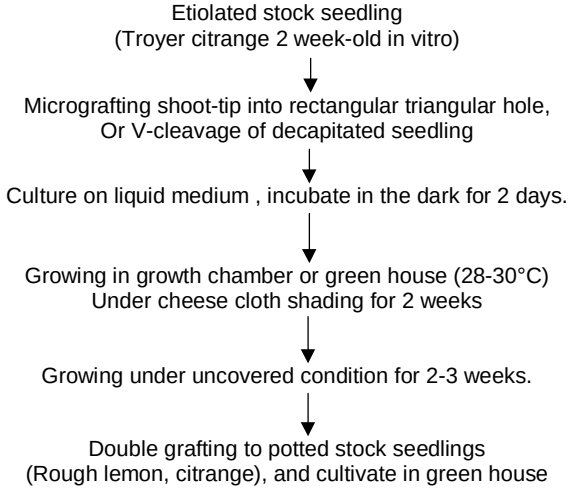
- **Elimination of inoculum sources.** Rouging infected banana plants when symptoms first appear and removing healthy-looking plants surrounding the infected stock are important measures for field sanitation. The phytosanitary procedures have been effective in Australia where strong government legislation has resulted in excellent control for bunchy top disease. CMV has a wide range of host plants. The avoidance of interplanting with CMV-susceptible solanaceous, cucurbitaceous and legume crops, and control of susceptible weeds are important for eradication of inoculum sources.

Virus-free nursery system

In order to control the systemic virus and greening disease of citrus, the present national scheme of citrus budwood certificate program was initiated, and conducted under a joint program in Taiwan since 1983.

- **Micrografting for obtaining pathogen-free citrus foundation stocks.**

An improved method of shoot-tip micrografting (STG) was developed by using triangle-hole cut instead of inverted T cut described by Murashige *et. al* (1972). The STG program and health indexing were conducted in the Plant Virology Laboratory, National Taiwan University. The outline of STG schedule is as follows:



The solid and liquid media were prepared following Murashige *et. al*

- **National scheme of pathogen-free nursery system.**

Pathogen-free Citrus Foundation Stocks. The stocks are produced by means of improved method of shoot-tip grafting and heat-therapy in the Plant Virus Laboratory, National Taiwan University (PVL / NTU). The stocks indexed as pathogen free by PVL / NTU are transferred to the Pathogen-free Citrus Foundation Stock Collection in screenhouses of the Chiayi Agricultural Experimental Station of Taiwan Agricultural Research Institute (CAES / TARI).

↓

Scion-Propagation Parent Trees. Pathogen-free scion woods are multiplied in scion-propagation trees derived from foundation stocks in the foundation screenhouse of CAES / TARI.

↓

Pathogen-free Citrus Seedlings. Pathogen-free seedlings are produced by using pathogen-free scions derived from the parent trees in the Healthy Nursery Screenhouses operated by each branch office of the Taiwan Fruit Marketing Cooperative.

↓

Citrus Growers. The healthy citrus seedlings are transplanted to the orchards protected by integrated control measures.

References

Burns, T.M.; Harding, R.M.; Dale, J.L. The genome organization of banana bunchy top virus: Analysis of six DNA components. J. Gen. Virology 76, 1995. pp. 1471-1482.

Diekman, M.; Putter, C.A.J. FAO / IPGRI Technical Guidelines for the Safe Movement of Germplasm. No. 15. Rome, Italy: FAO / IPGRI, 1995.

Lockhart, B.E.L. Purification and serology of a bacilliform virus associated with banana streak disease. Phytopathology 76, 1986. pp. 995-999.

Matsumato, T.; Wang, M.C.; Su, H.J. Studies on Likubin. In: Proc. 2nd Conf. IOCV, 1961. pp. 121-125.

Musharam, A.; Whittle. Stem pitting strains of citrus tristeza virus in Indonesia. In: Proc. 11th Conf. IOCV, IOCV. Riverside, 1991. pp. 150-155.

Roistacher, C.N.; Morens, P. The worldwide threat from destructive isolates of citrus tristeza virus – a review. In: Proc. 11th Conf. IOCV, IOCV Riverside, 1991. pp. 7-19.

Soomro, M.H.; Khalid, S.; Aslam, S. Outbreak of banana bunchy top virus in Sindh, Pakistan, EAO Plant Prot. Bull. 40, 1992. pp. 95-99.

Su, H.J. A tristeza virus strain causing dwarfing of pummelo and grapefruit. In: Proc. Intern. Soc. Citriculture Vol. 1,1981. Pp. 423-430.

Su, H.J.; Chen, C.N. Implementation of IPM of citrus virus and greening (likubin) diseases. In: Integrated Control of Plant Virus Diseases. FFTC Supplement No. 1, 1991. pp. 3-11.

Su, H.J.; Chu, J.Y. Modified technique of citrus shoot-tip grafting and rapid propagation method to obtain citrus budwoods free of citrus viruses and likubin organism. In: Proc. Int. Soc. Citriculture. Vol. 2. 1984. pp. 332-334.

Su, H.J.; Hung, T.H.; Tsai, M.C. Recent developments in the detection citrus greening disease. In: Proc. 6th Inter. Asia Pacific Workshop on "Integrated citrus health management". Kuala Lumpur, Malaysia, 1991. pp. 24-30.

Su, H.J.; Hung, T.H.; Wu, M.L. Preparation and application of diagnostic DNA probe on the fastidious bacteria causing citrus greening. In: Proc. Symposium on Research and Development of Citrus in Taiwan, 1995. pp 167-176.

Su, H.J.; H.J.; Hung, T.H.; Wu. M.L. First report of banana streak virus infecting banana cultivars (*Musa spp.*) in Taiwan. Plant Disease 81, 1997. pp. 550.

Su, H.J.; and Tsai, M.C. The etiological nature and transmission of fruit virus and virus-like diseases in Taiwan. Integrated Control of Plant Virus Diseases. FFTC Supplement No. 1. 1991. pp. 83-87.

Su, H.J.; Wu, R.Y.; Tsao, L.Y. Ecology of banana bunchy-top virus disease. In: Proc. Intern. Symposium on Recent Development in Banana Cultivation Technology, Taiwan, 1993. pp. 308-312.

Sun, S.K. Studies on the bunchy top disease of bananas. Spec. Publ. Coll. Agric. Taiwan Univ. 10: 1961. pp. 82-109.

Tsai, M.C.; Su, H.J. Development and characterization of monoclonal antibodies to citrus tristeza virus (CTV) strain in Taiwan. In: Proc. 11th Conf. IOCV, IOCV Riverside, Taiwan, 1991. pp. 46-50.

Tsai, M.C.; Su, H.J.; Garnsey, S.M. Comparative study on stem-pitting strains of CTV in Asian countries. In: Proc. 12th Conf. IOCV, IOCV Riverside, Taiwan, 1993. pp. 16-19.

Wu, J.L.; Hung, T.H.; Su, H.J. Strain differentiation of cucumber mosaic virus associated with banana mosaic disease in Taiwan. Ann. Phytopathol. Soc. Jpn. 63, 1997. pp. 176-178.

Wu, R.Y.; Su, H.J. Purification and characterization of banana bunchy top virus. J. Phytopathol. 128, 1990. pp. 153-160.

Wu, R.Y.; Su, H.J. Production of monoclonal antibodies against banana bunchy top virus and their use in enzyme-linked immunosorbent assay. J. of Phytopathology 128, 1990. pp. 203-208.

Wu, R.Y.; Su, H.J. Regeneration of healthy banana plantlets from banana bunchy top virus-infected cultured at high temperature. Plant Pathology 40, 1991. pp. 4-7.

Yeh, S.H.; Su, H.J.; Chao, Y.C. Genome characterization and identification of viral-associated DNA component of banana bunchy top virus. Virology 198, 1994. pp. 645-652.

Production and Cultivation of Virus-free Banana Tissue-cultured Plantlets in Taiwan

Shin-Chuan Hwang
Taiwan Banana Research Institute

Hong-Ji Su
Department of Plant Pathology
National Taiwan University

Banana is one of the most important fruit crops in many tropical and subtropical countries. Commercial cultivation of banana (Cavendish AAA) in Taiwan, involving a large number of small growers, is mainly production of fresh fruit for local consumption and export to Japan. With annual crop system, farmers traditionally use sword suckers as planting materials. Each mother plant supplies about 1-2 suckers during the planting season from March to May. Inevitably, many important diseases including viruses, Fusarium wilt pathogen, nematodes, and others are readily transmitted through planting suckers from one crop cycle to the next.

Fusarium wilt of Cavendish bananas caused by race 4 of *Fusarium oxysporum* Schl. f. sp. *cubense* (E.F. Smith) Snyder & Hensen, is the most serious problem affecting banana production in Taiwan. The solution identified and utilized was an economical replanting rotational system with paddy rice. The key was propagating cheap and clean planting materials through tissue culture (TC). Fusarium wilt was the primary reason for the development of meristem culture technique for mass propagation of disease-free banana plantlets for commercial planting in 1983. Since then, micropropagation of bananas for both the rapid production of planting materials and the storage and transfer of germplasm has become a common practice in Taiwan.

Many viruses are known to infect banana, causing serious losses in many countries. Viruses are usually spread from plant to plant by specific vectors. However, long distance and in-between crop cycle infestation is often spread through the use of vegetative planting materials. It is also known that virus can be readily transmitted through the tissue culture process, thus, to produce virus-free plantlets, source plants for tissue culture must be virus free. Virus indexing technology forms the most essential component in a virus-free seedling production program.

Prior to 1990, identification and detection of virus in bananas was based largely on symptomatology and electron microscopy. However, results from these methods were inaccurate and unreliable. In the last decades, advances in biotechnological studies on banana viruses at the Department of Plant Pathology National Taiwan University have led to the development of reliable and sensitive methods for detection of viruses. Implementation of such modern virus indexing technique in the banana micropropagation system in Taiwan has greatly improved the efficiency of tissue culture process and minimized the risk of virus being distributed through TC plantlets.

Banana Viruses and Detection Methods in Current Use

At present, viruses that have been identified as pathogens on *Musa* spp. are abaca mosaic potyvirus (AbMV), banana bract mosaic potyvirus (BBMV), banana bunchy top virus (BBTV), cucumber mosaic cucumovirus (CMV), and banana streak badnavirus (BSV). Among these five viruses, AbMV was reported to infect only abaca (*Musa textilis*) which produces the Manila hemp from the Philippines, while the other four cause yield loss of economic importance on various banana cultivars.

To establish a program for producing virus-free banana TC plantlets, one has to know the kinds of viruses present in the country and to select the suitable method for virus detection. The "Technical Guidelines for the Safe Movement of *Musa* Germplasm" published by FAO / IPGRI (1995) provides information on the symptoms, host range, geographical distribution, transmission, and detection of banana viruses in Taiwan. The short and concise guidelines is useful for people involved in the banana tissue culture work. Excerpt from this technical guidelines is shown in Table 1.

A range of techniques can be used to detect and identify banana viruses including symptomatology, electron microscopy, indicator plants, serology and nucleic acid hybridization. Each method has certain advantages and disadvantages. Modern serological detection techniques can be highly sensitive to detect known viruses, but are also highly specific that for reliability maybe complicated by the occurrence of a wide degree of serological diversity of a virus. For instance, a recent study at the National Taiwan University showed that polymerase chain reaction (PCR) technique was able to detect six strains of BBTV isolates collected from Taiwan and Malaysia, but two of them – latent and Malaysia strains - could not be detected by the enzyme-link immunosorbent assay (ELISA).

Currently, three banana viruses have been reported in Taiwan. BBTV and CMV are widespread in banana production areas and occasionally cause diseases of epidemic proportions. More recently, BSV was found in few plants of Mysore (AAB) grown in the germplasm collection of the Taiwan Banana Research Institute (TBRI). Although BSV has been found in only few plants of Cavendish cultivars, further studies indicated that Cavendish cultivars were more susceptible to BSV than Mysore, and pose a great threat to our banana industry. In Taiwan, ELISA method was developed for the detection of BBTV and CMV, while PCR method for BBTV and BSV.

Sampling technique is also an important consideration when assaying for banana viruses. Virus concentration and symptom expression can vary considerably between plants and even between leaves of the same plant. Studies in Taiwan demonstrated that concentration of BBTV was higher in younger leaves, and decreased with increasing age of the leaf. In contrast to BBTV, BSV was present in higher concentrations in older leaves, and lower in younger leaves. Accordingly, samples for BBTV detection should be taken from younger leaves, while for BSV detection from older leaves.

Production of Virus-free TC Banana Plantlets in Taiwan

A total of 26 million banana plantlets has been produced for commercial planting in Taiwan since the TC program was started in 1983 (Table 2). The Cavendish cultivars used for propagation are Giant Cavendish,

Table 1. Viruses of *Musa* spp.

Virus	Geographical Distribution	Host Range	Transmission Vectors	Serological Detection
Abaca mosaic (AbMV)	Philippines	Abaca (*Musa textilis*) *Canna indica* *Marantha arundinacea*	Aphids *Rhopalosiphum maidis* *Aphis gossypsii*	ELISA
Banana bract mosaic (BBMV)	Philippines, India Sri Lanka	*Musa* spp. & cultivars	Aphids *A. gossypsii* *Pentalonia nigronervosa* *R. maidis*	ELISA
Banana bunchy top (BBTV)	Cosmopolitan except for Latin America	*Musa* spp. & cultivars *Aphinia formosana* *Hedychium coronarium*	Aphid *P. nigronervosa*	DNA probes ELISA PCR
Banana mosaic (CMV)	Cosmopolitan	*Musa* spp. & cultivars Extremely wide host range over 800 spp.	Aphids *A. gossypsii* *R. maidis* *R. prunifoliae* *Myrus perisicae*	ELISA
Banana streak (BSV)	Cosmopolitan except for Latin America	*Musa* spp. & cultivars	Citrus mealybug *Planococcus citri*	ISEM PCR

Table 2. Quantity of banana meristem plantlets propagated from 1983-1998.

Year	Cultivar Giant Cavendish	Tai-Chiao No.1	Tai-Chiao No.2	Total
1983-1986	2,267	0	0	2,267
987	2,265	0	0	2,265
988	2,104	0	0	2,104
989	1,319	0	0	1,319
990	613	1,388	0	2,001
991	641	2,052	112	2,805
992	1,154	918	167	2,239
993	447	73	144	664
994	538	47	479	1,064
995	986	93	531	1,610
996	1,088	49	370	1,507
997	2,505	492	451	3,448
998	2,244	312	445	3,001
Total	18,171	5,424	2,699	26,294

(Unit: x 1,000)

Tai-Chiao No.1, and Tai-Chiao No.2. Tai-Chiao No.1, a somaclone derived from Giant Cavendish known to be resistant to race 4 of Fusarium wilt, was released in 1991 for commercial planting in the infested areas. Tai-Chiao No.2, an introduced semi-dwarf cultivar, was released for planting in 1992. Application of TC technique for banana plantlet production benefited our banana growers not only through the production of disease-free planting materials but also in the rapid multiplication of new varieties.

The procedure for commercial micropropagation of banana plantlets consists of four stages: culture initiation, bud multiplication, plantlet regeneration, and acclimatization in the nursery. Taiwan has an annual production program aimed at producing millions of plantlets, where hundred thousands of suckers are required for culture initiation each year. Initial procedures required that suckers directly obtained from the field be subjected to virus indexing and only those proven to be virus free are used for culture initiation.

However, virus indexing for such a large number of samples is time consuming and costly. To cut on cost and time, virus-free stock foundations needed to supply virus-free material for culture initiation purpose were

established. The establishment and management of the virus-free stock foundation at TBRI is described below.

1. *Facility*

   A 32-meshed screenhouse measuring 40 m long x 40 m wide x 3.5 m high with double doors at the entrance. The screenhouse is insect-proof and vector-free.

2. *Stock preparation*
   - For each variety, about 10-20 plants showing vigorous growth, producing excellent bunches and true-to-type variety are selected from commercial orchards. Each plant is regarded as a 'line'.
   - For each line, 1-2 suckers are taken to the TC laboratory, subjected to virus indexing using ELISA and PCR double tests for BBTV and BSV, and ELISA test only for CMV. BBTV, BSV, and CMV are known to be present in Taiwan. The suckers showing negative reaction to all these viruses are further multiplied with tissue culture method to produce five plantlets / suckers, while those showing positive reaction to any one of these viruses are discarded.
   - All the plantlets produced for each line (about 5-10 plantlets / line) are subjected to virus indexing for the second time. Plantlets of virus-free line are used as the stock plants for planting in the foundation screenhouse.

3. *Management*
   - Plantlets are planted in a growth medium free from Fusarium wilt pathogen and consisting of sawdust and compost (4:1 v / v). Irrigation is through ground water irrigation.
   - Stock plants in the screenhouse are regularly observed for symptoms of virus and Fusarium wilt. Virus indexing is conducted at a 3-month interval. Infected plants are eradicated immediately. Chemical sprays for controlling banana diseases and insects are conducted regularly along with proper fertilization to promote sucker formation.

For culture initiation, suckers are exclusively obtained from stock foundation. At this stage, virus indexing is done on one sucker for each line. Virus indexing is not necessary at bud multiplication and plantlets regeneration stages until the plantlets reach the final nursery stage.

The commercial nurseries are preferably established in non-banana growing areas. Construction and management of nurseries is basically the same as stock foundation establishment mentioned above, except for indexing for viruses which is done only for a certain amount of samples of plantlets. The nursery should be insect-proof, vector-free, and strictly follows all sanitary procedures to prevent virus reinfection or introduction of other pathogens. Plantlets are inspected regularly for off-type mutants and for abnormal plantlets showing symptoms of viral infection.

Cultivation of Virus-free Banana TC Plantlets

The use of TC plantlets for planting has many advantages. A TC plantlet is cheaper and easier to propagate and transport, has a higher survival rate in the field, reduces cost for controlling foliar diseases by 50%, uniformity in growth makes control of flowering and harvesting possible, and significant increase in yield and fruit quality. However, with the

Table 3. Comparison of CMV incidence between suckers and plantlets after planting in banana orchards.

Variety	Kind of planting material [1]	No. of plants surveyed [2]	CMV (%)
Giant Cavendish	TCP	16,087	2.9
	S	1,660	0
Tai-Chiao No.1	TCP	3,072	12.0
	S	5,554	0.2
Tai-Chiao No.2	TCP	13,350	0.4
	S	2,340	0

[1] S:Sucker, TCP:Tissue Culture Plantlet
[2] The survey was made in July 1995, 2-3 months after planting

introduction of TC plantlets for planting, new problems arise. There are strong evidences indicating that plantlets are more susceptible to CMV than suckers (Table 3). Outbreaks of CMV in some orchards growing TC plantlets registered an infection rate of up to 65%. Incidence of CMV is usually associated with orchards with poor weed management practices or in communities growing vegetable crops like bean, cucumber, pepper etc. These vegetables are known host of CMV. At early growth stage, plantlets are more sensitive to herbicide than suckers. Too high labor costs make weed control by hoeing impossible and excessive use of herbicides inevitably causes varing degrees of damage to the plant.

Also, mature plants grown from plantlets tend to develop 'floating mat' and are prone to toppling over after shooting.

To overcome these problems, the following guidelines are recommended for cultivation of virus-free plantlets with special emphasis on viral control:

- To prevent 'floating mat' problem, planting of plantlets should be 10-15 cm below the soil surface.
- To reduce herbicide damage at early growth stages of the plantlets, plastic cloth mulching for weed control is recommended. Plastic mulching is effective, economical, and promotes growth of banana plants.
- The fertilization program for plantlets can follow the standard recommendation for commercial banana plantations except for plantlets in which the first application of fertilizer must be made earlier. While suckers receive fertilizer about one month after planting, plantlets should be fertilized about 10 days after planting. Occassional occurrence of heart rot disease of TC plantlet, caused by boron and calcium deficiency was observed in Taiwan. In orchards with too sandy and acidic soil, application of borax (2kg/ha) and liming is recommended for controlling the heart rot disease.

Integrated Control of Viral Disease

- CMV is transmitted through weeds and many vegetables like bean, cucumber, pepper, and tomato by a number of aphids. Planting of plantlets must be avoided in banana orchards near areas growing these vegetables. In vegetable-growing areas known to have high

CMV incidence, use suckers instead of plantlets for planting. Suckers are more tolerant to CMV infection. Suckers must be obtained from the orchards free from viral symptoms. Also, good management of weeds effectively reduces aphid populations.

- Use larger plantlets for planting - Greenhouse studies have shown that tolerance to BBTV and BSV is correlated with the size of banana TC plantlet. For example, when 5-10 cm tall plantlets were inoculated with BSV, the infection rate reached 75% and incubation period averaged 22 days; the corresponding figures for 30 cm and 50 cm tall plantlets of were 50%/80 days and 25%/135 days, respectively.
- Selection of planting time - In Taiwan, most banana plantlets are planted in the fields during the period from March to May and are harvested in the same period the subsequent year. Field surveys in the last three years revealed that CMV incidence was consistently high in March (8.0%), moderate in April (3.3%), and lowest in May (1.9%)(Chao, unpublished). To reduce viral infection, planting of TC plantlets after mid-April is recommended.
- Soil mulching with shining plastic cloth - Hanging or soil mulching with shining plastic cloth in the field to repel aphid has proven to be effective in controlling viral diseases in many crops. Experiments conducted at TBRI showed that CMV incidence was decreased from 52% in non-mulching plots to 11.2% in the shining plastic mulching plots (Chao, unpublished). Mulching done before planting also served as a weed control strategy.
- The most fundamental rules for viral disease control is that early detection and roguing of infected plants can not be overlooked in the integrated control program. Infected plants are sprayed with kerosene to kill aphids prior to destruction by herbicide.

Conclusion

Banana production is increasingly suffering losses due to viral diseases in many banana growing countries. At present, five viruses infecting *Musa* spp. have been reported: AbMV, BBMV, BBTV, CMV, and BSV. These viruses are transmitted through vegetative planting material. Successful control of viral diseases should start out with the virus-free planting material. The solution is the efficient propagation of cheap and clean planting materials through tissue culture.

In Taiwan, Fusarium wilt is the most serious disease affecting banana production. As a systemic disease, Fusarium wilt pathogen is spread through suckers, the conventional planting material used by banana growers in Taiwan. In 1983, a tissue culture program for mass production of disease-free banana plantlets for commercial planting was developed. A total of 26 million TC plantlets has been released to banana growers for the period 1983-1998. Advances in biotechnological studies on banana viruses in the last decade in Taiwan led to the development of sensitive, reliable methods for virus detection. ELISA was employed for detecting BBTV and CMV, and PCR was used for BBMV and BSV. The four banana viruses known to be present in Taiwan. These methods were implemented recently in the banana TC plantlet production system to improve the virus-free quality of seedlings.

For reliable detection of banana viruses using ELISA and PCR,

knowledge of the serological diversity of a virus is important. For BBTV and CMV, a few distinctive strains have been reported, but little is known about the serological properties of BBMV and BSV, thus, further study is needed.

Although tissue culture is believed to eliminate the risk of plantlets carrying fungal, bacterial, and nematode pathogens and insect pests of bananas, it could still carry virus pathogen if the source plants used for culture initiation were virus-infected. In a country where banana viruses are always present, suckers taken from commercial plantation and used directly for culture initiation should be prohibited. To supply large numbers of suckers for TC program each year, virus-free stock foundations were set up in Taiwan. The stock plants raised in insect-proof, vector-free screenhouse are inspected constantly for virus symptoms and subjected to virus indexing regularly. The nursery for acclimatization of plantlets should also be under insect-proof, vector-free conditions where proper sanitation is enforced. For plantlets showing symptoms suspected of viral infection, regular inspection for off-type mutants and virus indexing are necessary before plantlets are released to the growers.

To overcome problems on the herbicide damage and viral infection common to field - planted TC plantlets, planting of plantlets at 10-15 cm and soil mulching with plastic cloth for weed control are recommended. For cultivation of plantlets, application of fertilizer should start at 10 days after planting. Integrated management recommended for controlling viral diseases in Taiwan includes use of larger plantlets for planting, selection of suitable planting time to escape viral infection, use shining plastic cloth mulching to repel aphids, avoid planting of plantlets beside vegetable crops known to be hosts of CMV, and early detection and roguing of the infected plants.

References

Diekmann, M.; Putter C.A.J. FAO / IPGRI Technical Guidelines for the Safe Movement of Germplasm, No.15. Rome, Italy. FAO / IPGRI, 1995.

Fan, L.Y. Causal virus and transmission ecology of banana streak. M.S. Thesis, Graduate Institute of Plant Pathology and Entomology, National Taiwan University, Taiwan. ROC, 1998.

Hwang, S.C.; Chen, C.L.; Lin J.C.; Lin, H.L. Cultivation of banana using plantlets from meristem culture. HortScience 19: 231-233, 1994.

Hwang, S.C. Ecology and control of fusarium wilt of banana. In: Plant Prot. Bulletin 27: Taiwan , 1984. pp. 233-234..

Hwang, S.C.; Ko, W.H.; Chao C.P. GCTCV-215-1: A promising Cavendish clone resistant to race 4 of *Fusarium oxysporum* f. sp. *cubense*. In: Plant Prot. Bulletin 36: Taiwan, 1994. pp. 281-291.

Ko, W.H.; Chen S.P.; Chao C.P.; Hwang S.C. 1997. Etiology and control of heart rot of banana tissue-cultured plantlets. In: Plant Pathology Bulletin 6: Taiwan, 1997. pp. 31-36.

Stover, R.H. Banana, plantain and abaca diseases. Surrey, England: C.A.B. Kew, 1972. 316pp.

Su, H.J.; Hwang, S.C.; Ko, W.H. Fusarium wilt of Cavendish banana in Taiwan. In: Plant Disease Bulletin 70: Taiwan, 1994. pp. 814-818.

Su, H.J.; Hung, T.H.; Wu, M.L. First report of banana streak virus infecting banana cultivars (Musa spp.) in Taiwan. Plant Disease Bulletin 81, Taiwan, 1997. pp. 550.

Tsao, L.Y. Biological and molecular characterization of banana bunchy top virus strains and their ecology. Ph.D. Thesis, Graduate Institute of Plant Pathology and Entomology, National Taiwan University, Taiwan, ROC, 1998.

Wu, R.Y.; Su, H.J. Production of monoclonal antibodies against banana bunchy top virus and their use in enzyme-linked immunosorbent assay. J. Phytopath. Bulletin 128: Taiwan, 1990. pp. 203-208.

Wu, M.L.; Hung, T.H.; Su, H.J. Strain differentiation of cucumber mosaic virus associated with banana mosaic disease in Taiwan. Ann. Phytopathol. Soc. 63 Jpn. , 1997. pp. 175-178.

Viruses of Banana and Methods for Their Detection

J. E. Thomas
Queensland Horticulture Institute
Department of Primary Industries
Australia

Introduction

A number of viruses affect banana and plantain (*Musa* spp.) around the world. In this presentation the following viruses will be considered:

Abaca mosaic virus (AbaMV)
Banana bract mosaic virus (BBrMV)
Banana bunchy top virus (BBTV)
Banana dieback virus (BDBV)
Banana potexvirus
Banana streak virus (BSV)
Cucumber mosaic virus (CMV)

Bananas and plantains are vegetatively propagated crops, and as such, virus contamination of planting material is a significant issue. Once infected, a plant and its progeny will remain infected thereafter. Although tissue culture is an effective means of freeing planting materials of most pests and diseases, viruses are usually very difficult to eliminate.

However, even though some viruses have only been recently been discovered or characterized, functional detection methods are available for all of them. Good summaries of the characteristics, symptoms, and accepted indexing methods for most of the above viruses can be found in Diekmann and Putter (1996).

Detection Methods

Symptoms. While symptomatology is the easiest of the detection methods, it is not always reliable. In some cases, classic symptoms may be displayed allowing fairly certain identification of the virus involved. However, mixed infections are a frequent occurrence and in these cases symptoms are not always clear. Symptoms caused by different viruses can be similar and infected plants may even be symptomless.

BBTV. Typical symptoms of BBTV-infection are diagnostic. These include erect growth habit with yellow, curled-up margins bunched leaves. Dark-green dots and dashes on the lamina, petiole, pseudostem and bract tips are added characteristic. However, mild strains of BBTV are known to occur in Taiwan and infected plants display few, if any, of the above symptoms.

BSV. Symptoms can vary widely but usually consist of chlorotic or necrotic streaks and flecks. Some strains can also cause necrosis of the heart leaf and pseudostem. Symptomless infections are common and frequently come and go throughout the growing season, presumably due to environmental conditions. In the past, BSV-symptoms were often confused with those caused by CMV.

CMV. Usually, symptoms consist of mosaic patterns, chlorosis or necrotic streaks and flecks, and sometimes necrosis of the cigar leaf and pseudostem.

BBrMV. Distinctive dark streaks and mosaics on the flower bracts are characteristic of BBrMV. When the outer leaf sheaths are removed, streak and margins are often seen on the pseudostem. These streaks can also be seen on the petioles. Leaf symptoms, when present, usually consist of chlorotic spindle-shaped lesions. Early symptoms of infection include chlorotic patches with rusty red borders and are very similar to those caused by AbaMV.

Banana potexvirus. The symptomatology of the banana potexvirus is uncertain. In many cases, symptomless infections occur, and mixed infections with other viruses are common. In a few instances, single infections have been associated with fine chlorotic striations and streaks.

AbaMV. Broad yellow or pale green bands and mosaic across the width of the lamina is typical in AbaMV. In older infections, chlorotic, orange or dark-green streaks may be present on the midribs and petioles. Early infections, however, can be confused with those caused by BBrMV.

BDBV. This virus causes symptoms of leaf crinkling, necrosis, and cigar leaf dieback.

Sampling. Sampling procedures and strategies are important, often overlooked aspect of virus indexing: viruses can only be detected in tissues they are actually in.

BBTV is a phloem-restricted virus, and in infected leaves the concentration of virions is up to five times greater in the midrib compared to the lamina tissue. Virus levels are also higher in the younger leaves. When a stool becomes infected late in the cropping cycle, there may be a delay before all suckers and meristems become infected. To effectively index a banana stool for BBTV, it is necessary to sample the youngest 1-3 leaves (preferably the midribs) from the mother plant and all available suckers. These can be pooled provided the assay has sufficient sensitivity. For *in vitro* plants, all plantlets must be sampled, as a small proportion of virus-free plants can arise from an infected line during culture.

By contrast, the distribution and concentration of CMV and BSV can be quite erratic. Studies have shown that the levels of CMV vary, both in different parts of a leaf and between different leaves on a plant. BSV is notoriously erratic in its distribution within infected

plants and in its detectability throughout the year. In field trials in Australia, in plants known to be BSV-infected, between 5% and 80% of random leaf samples from the youngest leaves contained detectable virus by ELISA. At no stage did every plant indexed positive (Geering, Daniells & Thomas, unpublished).

The banana potexvirus was shown to be fairly evenly distributed and at a uniform concentration throughout the midrib and lamina of all leaves (M Sharman and JE Thomas, unpublished).

Serological Assays. Various forms of serological assays are currently available for all seven of the banana viruses discussed. When suitable antisera are available, the most sensitive and efficient practical assay for banana virus indexing is enzyme-linked immunosorbent assay (ELISA). Specific polyclonal and monoclonal antibodies are available for BBTV, BSV, CMV, BBrMV (Diekmann and Putter, 1996) and the banana potexvirus (CF Gambley and JE Thomas, unpublished; M-L Caruana, personal communication). BDBV polyclonal antiserum has been prepared (J d'A Hughes, personal communication) and antisera to the sugarcane mosaic virus subgroup of potyviruses are effective for the detection of AbaMV.

For the detection of BBTV, Triple Antibody Sandwich (TAS)-ELISA was the preferred method when compared to dot immunobinding assays and amplified ELISA (Geering and Thomas, 1996). ELISA tests are also available for all other banana viruses discussed here.

In most cases, the available antisera have broad enough specificity to detect the range of virus isolates present. CMV exists as two major serogroups, some antisera being serogroup specific, others reacting to both. The latter types need to be used for virus indexing, as both serogroups occur in banana. Potyvirus group-specific monoclonal antibodies are commercially available though not all isolates of BBrMV react with these. Their usefulness as a routine diagnostic tool for banana indexing is therefore questionable. Not all isolates of BSV can be detected by ELISA using the currently available antisera.

Immunosorbent Electron Microscopy (ISEM) can be used when antisera are not of sufficient quality for ELISA, when only a small number of samples are to be indexed or screened for a number of viruses simultaneously.

PCR. The most significant recent advance in virus indexing has been the advent of the Polymerase Chain Reaction (PCR). This is a very sensitive assay, but not without its problems. Components of banana sap can inhibit the reaction and also, some knowledge of the virus' genome is required. PCR has been used to detect AbaMV, BBTV, BBrMV, BSV, and CMV from total nucleic extracts of infected banana. Standard PCR (and reverse transcription -RT- PCR for RNA viruses) are limited in their usefulness as routine diagnostic assays due to the tedious sample preparation required and the expense of the reagents. Modifications such as immunocapture steps and multiplex assays can overcome some of these problems.

We have developed an immunocapture, multiplex RT PCR for the simultaneous detection of BBTV (a DNA virus), BBrMV, and CMV (RNA viruses) in a single test. The method involves trapping virions

from an ELISA sap extract in a tube coated with a mixture of virus-specific antibodies. After an RT step for the RNA viruses, PCR is performed with primers to all viruses in one reaction tube. The products are run on standard agarose electrophoresis gels or in a microplate detection system. Due to the sensitivity of the system, particular care needs to be taken to avoid cross contamination. This test has been used to detect a wide range of isolates of each virus and can accommodate additional viruses as reagents become available.

BSV - The difficult virus. Not only is BSV irregularly distributed in plants over time and space, it is also genomically and serologically very heterogeneous. This makes detection with a virus-specific assay very difficult. The integration of BSV sequences into the banana genome also makes detection by PCR problematic.

At this stage, detection is most efficiently achieved by checking for the presence of virus particles by ISEM of partially purified extracts - a time consuming process. The antiserum used was prepared by Dr. B. Lockhart from a mixture of over 30 different isolates of BSV and it detects all known strains of BSV in ISEM, but not ELISA.

Current and Future Recommendations

The choice of indexing procedure depends partly on the facilities available at the indexing center. Plants should always, where possible, be inspected for symptoms. This can provide good evidence for virus infection and can also indicate the presence of viruses that are either unknown or not being specifically tested for. In most cases, ELISA is the indexing method of choice, combining ease of use, good sensitivity, high throughput and minimal technical facilities. Antibodies are commercially available for many of the viruses. The difficulties arise with BSV, as the method of choice requires an ultracentifuge and an electron microscope. ELISA can be used as an alternative, but in the knowledge that some isolates could be missed. Current indexing recommendations are found in Diekmann and Putter (1996) and protocols for detection of BBTV and BBrMV by ELISA are shown in the appendix.

Where facilities are available, the multiplex IC-PCR could replace several ELISA tests, minimizing the use of antibodies and increasing the throughput of samples. This assay has the advantage of being able to incorporate tests for additional viruses as they become available.

ELISA Protocol for the Detection of Banana Bunchy Top Virus

1. Coating of Polystyrene Plate

- BBTV polyclonal antiserum 6-6-94 bleed immunoglobulin fraction (Ig) diluted in carbonate coating buffer (#) to 5-10 mg / ml (For one ELISA plate, need 25-50 mg IgG in 5 ml)
- Add 50 ml / well.
- Incubate 2 - 3 hours at room temperature in incubation chamber[1]
- Wash 3 x 3 min with PBS-TÄ

2. Antigen

- BBTV samples and controls (both known healthy and diseased material, the mid-rib area is usually chosen) are ground in a mortar and pestle after cutting into thin sections, using a pinch of acid-washed sand (AJAX CHEMICALS) and cold 0.2 M potassium phosphate buffer, pH 7.4 + 0.5% sodium sulphite. The buffer should be added at the commencement of grinding as oxidation of the banana leaf material will occur when this is not done. The dilution rate of leaf material to buffer is 1 g / 4 ml.
- Brief low speed centrifugation of sap sample
- Add 50 ml supernatant / well
- Incubate 5°C overnight
- Wash 3 x 3 min with PBS-TÄ

3. Detecting

- BBTV monoclonal antibodies (Mab) 1 (2AB1-B12-D8) and Mab 2 (3B4-B12-A6) diluted in PBS-T + 5% skim milk powder. Tissue Culture supernatants used in the ratio 2ml Mab 1 : 0.5 ml Mab 2 : 2.5 ml PBS-T + 0.25 g skim milk powder. (For one ELISA plate)
- Add 50 ml / well

Buffer Recipes

(#)Carbonate Coating Buffer:

1.59 g Na_2CO_3)
2.93 g $NaHCO_3$) in 1 L solution pH 9.6.

* **PBS-T:**

PBS stock diluted by a factor of 5 (200 ml in 1 L), and add Tween 20 to a final concentration of 0.05% (0.5 ml / L)

5 X Phosphate Buffered Saline (PBS) Stock:
(5 X working concentration :- add 200ml 5 X PBS to 800ml H_2O)

40.0 g NaCl	)	
1.0 g KH_2PO_4	)	in 1 litre solution pH 7.4
14.5 g $Na_2HPO_4.12H_2O$	)	(adjusted with 3 M NaOH)
1.0 g KCl	)	

Diethanolamine Buffer:

97 ml diethanolamine - made up to *ca* 850 ml with dH_2O, adjusted with HCl to pH 9.8, then made up to 1 L. Used at the rate of 100 ml / well.

[1]**Incubation Chamber -** Place moist towels (2 or 3 sheets) in the bottom of a small plastic container with tight fitting lid. For each step of the ELISA, place the microtitre plate in this box to keep it in a humid environment to avoid drying out.

[2]**Conjugate -** We use commercially available antimouse at the recommended dilution.

[3]**Incubation Chamber -** Place moist paper towels (2 or 3 sheets) in the bottom of a small plastic container with a tight fitting lid. For each step of the ELISA, place the microtitre plate in this box to keep it in a humid environment to avoid drying out.

[4]**Wash -** First discard incubated sample, then fill plate with buffer, incubate for 3 minutes, discard buffer, and repeat twice. After the third wash, buffer is discarded and plate tapped upside down onto towel completely empty the wells of liquid.

[5]**Conjugate-** We use commercially available sheep antimouse or rabbit antimouse at the recommended dilution.

References

1. Diekmann, M. and Putter, CAJ. 1996 FAO / IPGRI Technical Guidelines for the Safe Movement of Germplasm; No.15. *Musa*. 2nd edition. Rome Food and Agriculture Organization of the United Nations, Rome / International Plant Genetic Resources Institute.
2. Geering, A.D.W. and Thomas, J.E. A comparison of four serological tests for the detection of banana bunchy top virus in banana. *Australian Journal of Agricultural Research* 47:403-412, 1996.

Virus and Virus - like Diseases of Banana and Citrus in Malaysia: Status and Control Strategies

Ong Ching Ang
Malaysian Agricultural Research and Development Institute (MARDI)
Kuala Lumpur, Malaysia

Lily Ong
ARC-Department of Agriculture
Sarawak, Malaysia

Abstract

At present, only seven insect-borne viruses have been reported on tropical fruit crops in Malaysia. Out of these, banana bunchy top virus (BBTV), cucumber mosaic virus (CMV), and banana streak virus (BSV) infect banana while citrus tristeza virus (CTV) infects citrus. These are recognized to be serious threat in the production of their respective host crops. In the case of BBTV, asymptomatic and symptomatic variants of BBTV have been isolated. To date, symptomatic variants of the virus have been recorded only in the state of Sarawak in East Malaysia while the rest of the country is still free of these variants. Similarly, several strains of CTV have also been recorded. However, CTV-D has not been isolated from pummelo. Although vectors of these viruses are present, aphid vector of BBTV, *Pentalonia nigronervosa*, and mealy bugs vector of BSV, *Planococcus citri*, are seldom found in abundance in banana plantings.

The only confirmed case of virus-like disease is greening disease of citrus caused by the greening organism (GO). Its psyllid vector, *Diaphorina citri*, are found in poorly managed orchards and on new flushes. This disease is also considered to be one of the major limiting factors in citrus production in the country.

Management strategies of these pathogens through quarantine, use of pathogen-free planting stocks, field eradication of inocula, isolation from sources of infection, chemical control and mild strain cross protection are discussed in this paper.

Introduction

Recent economic down-trend in Malaysia has affected the various sectors of the economy differently. While there is significant slowdown in the various industries, the agriculture sector has remained strong and robust. In fact, recognizing the importance of utilizing the nation's resources fully in this trying time, the government is encouraging more investment in food production. With this, there is a corresponding increase in the interest on agriculture. This includes banana and citrus as well as other crops such as durian, rambutan, guava, starfruit, mangosteen, papaya, and vegetable crops.

Although banana and citrus originated in Southeast Asia, major production areas of these important fruits are to be found outside the region. Production figures of these two crops within the region are comparatively

low. Climate, farm size, soil fertility, and pest and disease problems have been cited as some of the contributory factors.

Malaysia's hot and humid tropical weather is exceptionally conducive to the survival and spread of pests and pathogens. This is primarily due to the continuous pest and pathogen cycles and the abundance of host plants all year round. For instance, the continuous flushing of new shoots in citrus encourages the colonization of aphid and psyllid vectors. Status of virus and virus-like diseases of fruit crops in Malaysia including those of banana and citrus have been reviewed previously (Ong and Ting, 1976, Ong and Doon, 1988, Ong, 1995).

Banana and Citrus Production

Banana

Malaysia is the center of diversity of banana. More than 200 species of banana have been recorded. Most of them are edible, but a few are ornamentals while some are considered weeds. Banana is popularly grown throughout the country. The popular banana types are listed in Table 1. Large scale planting of banana started in 1984. Today, banana plantations producing mainly the Cavendish and the Berangan banana are found in Johor, Perak, and Pahang.

Banana is produced both for local consumption as well as for export. It features prominently in the dietary intake of the average Malaysian. It is the major crop of the country and is exported to Singapore, Hongkong, Middle East, and Western Europe.

Table 1. Local names of popular bananas grown in Malaysia.

Eaten Fresh	Cooked Banana
Pisang Mas (AA)	Pisang Nangka (AAB)
P. Berangan (AAA)	P. Raja (AAB)
P. Embun (AAA)	P. Tanduk (AAB)
P. Rastali (AAB)	P. Abu (ABB)
	P. Awak (ABB)

Citrus

Compared to banana, citrus is a minor fruit crop in Malaysia although it (mainly limau langat) was more extensively cultivated in the 50s and 60s. The decline in the citrus acreage has been attributed to Phytophthora disease. However, evidence suggests that GO and CTV might be the major causal factors. Presently, several states are actively carrying out citrus rehabilitation programs.

The most popular citrus is *Citrus suhuiensis*, locally known as limau langat. Other popular citrus are *C. reticulata* (Chinese mandarin), *C. sinensis* (sweet orange), *C. grandis* (pummelo), *C. aurantifolia* (lime), *C. madurensis* (calamondin) and *C. hystrix* (limau purut).

Status of Virus and Virus-like Diseases

Banana

Banana Bunchy Top Virus. Banana bunchy top disease was first discovered in Fiji in 1889 (Stover, 1972). Serious epidemics of the disease have since been reported in many major banana-growing countries. Today, it is one of the major constraints in banana production in the tropics as well as subtropics. Field spread is usually by the banana aphids, *P. nigronervosa*. However, in areas where strict certification is not observed, the virus is largely introduced through tissue culture plantlets as well as infected young suckers.

Malaysia has long been listed as a BBTV-free country. However, recent reports have shown the presence of asymptomatic as well as symptomatic variants of this virus. The presence of the symptomless variant of BBTV was first confirmed in 1991 by Prof. H.J. Su using monoclonal antibodies which could detect mild strains of BBTV (Ong, 1995 and Ong *et al*., 1996). Throughout the country, symptomless variant was found prevalent in Pisang Mas, Pisang Rastali, and Cavendish (Ong and Su, unpublished). Subsequent surveys conducted in Peninsular Malaysia, Sabah, and Sarawak failed to find any symptomatic variants of the virus. It was then concluded that only the symptomless variant of the virus was present in the country.

In mid-1995, Pisang Berangan plants showing typical banana bunchy top symptoms were found in the Agriculture Research Center, Semengok and in Bau, Sarawak. A state-wide survey of the virus using ELISA kit from AGDIA indicated that the symptomatic strains were present in four out of eight divisions in Sarawak and the infection rates varied from 0 to 33% (Table 2) (Eng, 1996). Immediate eradication of infected plants together with the low population of the aphid vector helped reduce the spread of the virus and kept its incidence at a low level in that state. To date, Sarawak is the only state with the symptomatic variants of BBTV. All other states in Malaysia remain free of these variants.

Table 2. Occurrence and distribution of banana bunchy top virus in Sarawak

Division	No. infected/No. tested	%
Kuching	4/17	24
Samarahan	2/101	2
Sri Aman	0/7	0
Sarikei	0/27	0
Sibu	1/37	3
Bintulu	4/12	33
Miri	0/19	0
Limbang	0/13	0

Cucumber Mosaic Virus. Another aphid-borne virus isolated from banana is cucumber mosaic virus (CMV). In banana, the virus causes banana mosaic or infectious chlorosis disease. It was first recorded in banana in New South Wales, Australia in 1929 (Stover, 1972). In Malaysia, the first incidence of the virus in banana was in 1971 (Ong, 1971). Incidence of CMV in banana

in Malaysia, however, has been low and sporadic. Hence, unlike in many tropical and subtropical regions, banana mosaic is not a limiting factor in banana production here. A possible reason is that the CMV strains infecting other crops do not cross infect banana readily.

CMV infects many other different plants and is transmitted by many aphid species in non-persistent manner. Many strains of the virus have been studied. In Taiwan, at least three strains distinguishable by specific monoclonal antibodies as well as symptomatology have been studied (Wu *et. al*, 1997).

Banana Streak. This virus, belonging to the badnavirus group, was first reported in the Ivory Coast in 1974 (Lassoudiere, 1974). In Malaysia, BSV-infected rastali banana was first recorded by Ong and Su in 1996 (unpublished). Infected leaves of rastali banana showing broken chlorotic lines to necrosis were collected from Johor and Perak. Confirmatory studies based on transmission using mealy bugs, *Planococcus citri* and PCR-mediated amplification confirmed the presence of the virus. Bacilliform virus particles were also detected in crude sap of the infected leaves using immunosorbent electron microscopy. Young infected Cavendish plants developed internal necrosis of the pseudostem and eventually died. However, recent survey showed that the incidence of BSV is very low.

Citrus

Tristeza. Citrus tristeza virus, a member of the closterovirus group, is the most destructive disease of citrus in the western hemisphere and has a worldwide distribution. It is the first fruit tree disease identified in Malaysia. Some of the susceptible citrus species include *C. reticulata, C. sinensis, C. aurantiflolia, C. microcarpa, C. hystrix, C. limon, C. swinglei, C. aurantium* and *C. grandis*. Many strains of the virus were detected based on leaf symptoms, stem pitting, and results of serological studies using monoclonal and polyclonal antobodies. However, the new strain, CTV-D which causes serious dwarfing of pummelo in Taiwan (Su, 1981), has not been isolated, although the common CTV strains were found to be graft transmissible to pummelo.

Up until recently, the presence of CTV is diagnosed by grafting on indicator plants. However, in 1991, Tsai and Su developed a protocol that can confirm serologically the presence of the virus. Using the protocol, CTV was confirmed to be prevalent in citrus plants. If CTV-infected mandarin cultivars were to exhibit symptoms, the tristeza disease would have long been considered to occur in epidemic proportion.

The prevalence of CTV among the mandarin citrus is disturbing because infected trees do not show symptom. It is commonly stated that the local mandarin cultivars are tolerant to CTV. This implies that infection by this virus does not reduce growth and yield. This has created a sense of complacency among the citrus nurserymen, growers, and extension officers resulting to little or no control measure being taken to prevent aphid colonization. Additionally, it is common to find CTV-infected seedlings being sold in the market especially the marcotted ones.

The importance of CTV in mandarin cultivars are often overlooked because the infected plants do not show symptoms. However, the synergistic effect of mixed infection of CTV with GO had been documented by Maitree

(1990). CTV- and GO-infected mandarin seedlings were found to express more severe symptoms than those singly infected. Such synergistic effect is well documented in other virus combinations. Hence, it is possible that most of the debilitating effect attributed to greening disease on mandarins in Southeast Asia may be accentuated and accelerated by the presence of CTV because the virus is very widely disseminated in the region. Of course, the other important consideration is that the symptomless mandarins become the sources of inocula not only to other healthy mandarins but also to other susceptible citrus species such as calamondin and lime.

Greening. Citrus greening disease was first reported in South Africa in 1947. A similar disease known as huang-lungpin (yellow shoot disease) was reported in 1943 in China (Matsumoto *et al*., 1961). In Malaysia, it was first confirmed in 1989 (Lim *et al*., 1989) although symptoms of greening disease had been observed much earlier (Tanaka and Ueno, 1978). Confirmation studies were done through chip grafting and transmission by the oriental citrus psyllid, *D. citri*. Later, typical ploemorphic organisms were observed in the sieve tubes of fruits with aborted seed (Garnier & Bove, 1989). More recently, with the help of Dr. Su, the causal agent was further confirmed by specific nucleic acid probes as well as by the polymerase chain reaction (PCR) amplification with specific primers (Su *et al*., 1995).

Most of the citrus cultivated in Malaysia are susceptible to greening disease. Pummelo which was long considered to be free from this disease has been found to be naturally infected with the disease first in the East (Teo, 1995) and then in Peninsular Malaysia (Ong & Su, unpublished).

Status of Insect Vectors. Aphids, psyllids and mealy bugs are the most important vectors of the banana and citrus pathogens discussed in this paper (Table 3). The many species of aphid vectors of CMV are prevalent in many crops and weeds in Malaysia. Fortunately, the incidence of the virus in banana is very low. The population of banana aphids and that of mealy bugs are also very low in local banana planting. This probably accounts for the low incidences of BBTV and BSV.

T. citricida, the major aphid vectors of CTV, are commonly found on citrus seedlings and also on new flushes of leaves. *Aphis gossipii, A. spireacolor,* and *T. aurantii* have also been found to colonize citrus plants and many other crops and weeds. The presence of these vectors have undoubtedly helped the rapid spread of CTV in citrus orchards.

The vector of GO, *D. citri*, is widely distributed in the lowland (Lim *et al*., 1989). However, under field conditions, the number of the colonies is rather small. This has been attributed to the general high rainfall in the country. This psyllid is more commonly found on *Murraya koeniggi*, locally known as curry bush or Kerupilai. In fact, this plant is a very good alternate food host of psyllid.

Control Strategies

Movement between Countries. International movement of *in vitro* banana plantlets and citrus either for commercial production or germplasm conservation have greatly increased. This has increased the risk of introducing exotic pathogens to the importing countries. In the context of the two crops

Table 3. Relative importance of insect vectors on Malaysian crops.

Insects	Fruits	Vegetables	Ornamentals	Rice
Aphids	+++	+++	+	-
Leafhoppers	-	-	-	+++
Plant hoppers	-	-	-	+
Mealy bugs	+	-	-	-
Whiteflies	-	++	+	-
Thrips	-	++	+	-
Psyllids	+	-	-	-

discussed here, banana bract mosaic virus and citrus tatter leaf virus are the two most dangerous exotic viruses that have not been reported in Malaysia and must, therefore, be prevented from being introduced by quarantine regulations. Importation of banana planting materials including those derived from tissue culture and citrus budwoods must be subjected to strict quarantine.

Although CMV, BSV, CTV, and GO are present in the country, quarantine measures are strongly recommended. This is to prevent the introduction of new strains which are still absent in the country such as CTV-D which infects pummelo.

Movement within Countries. Since symptomatic variants of BBTV have been found only in Sarawak, it is imperative that strict interstate quarantine be enforced to prevent the introduction of the variants to the rest of the country which are still free from them.

Use of Pathogen-Free Stocks. All commercial banana are propagated vegetatively either by using suckers or by tissue-culture plantlets. Similarly, commercial citrus are also propagated vegetatively by marcotting and bud grafting. These methods of propagation are ideally suited for the long distance spread of pathogens. It can be concluded that virus and virus-like pathogens infecting banana and citrus, like BBTV, BSV, and GO are mainly introduced in this way rather than by their respective vectors. Thus, the use of clean planting materials is the most beneficial and important control measure.

In Malaysia, the use of pathogen-free planting materials of citrus has been implemented recently. Pathogen-free foundation stocks of popular citrus cultivars are being kept in MARDI and Department of Agriculture, Sarawak. Budwoods from these plants are then used for the propagation of planting materials.

The control strategy using pathogen-free stocks is to dilute the effects of disease through the supply of large quantities of healthy planting material. An important feature of this approach is the maintenance of pathogen-free foundation materials which are protected from reinfection (Mink, 1981).

The use of pathogen-free planting materials implies that there should be published standards defining the criteria necessary for certification and ways to preserve them free of the pathogens both before and after planting. Nurserymen involved in the production of such materials should (1) use only virus-indexed budwood, (2) avoid rebudding, and (3) rogue abnormal trees in nursery rows. On the other hand, growers should (1) buy trees from nurseries that produce pathogen-free seedlings, (2) do not plant these materials with established trees that are virus-infected, and (3) be familiar with the symptoms of virus and virus-like diseases and be prompt in removing diseased trees (Mink, 1981).

Field Eradication of Inocula. In Sarawak, field eradication of BBTV-infected bananas and surrounding healthy plants within the radius of 200 m through injection of herbicides has been implemented. This was done when BBTV was first reported in the area (Eng, 1966). As a result of this systematic eradication, the number of BBTV-infected plants in the field have been greatly reduced. Allen (1978) listed four factors that influenced the success of a roguing program for the control of BBTV in Australia. These are incubation period of the virus, relative infection rate, detection efficiency, and eradication efficiency.

Isolation from Source of Infection. To derive maximum benefit from the use of pathogen-free planting materials, growers should ensure that these materials are not planted in or near the vicinity of established trees that are infected. If possible, old infected trees should be removed before planting these materials. Prompt removal of any newly infected seedlings will also help reduce the spread of the disease.

Chemical Control. To prevent infestation of vectors, healthy citrus seedlings should be sprayed with insecticides (Dimethoate or Malathion) especially during periods of new flushes.

Mild Strain Cross Protection. Since CTV and its vectors are prevalent in the country and there is lack of other effective control options, the preimmunizarion of healthy foundation stocks or mother plants with protective mild strains of the virus might provide a solution to the problem. Success of such protection has been reported in Brazil where the method is being used extensively to protect certain scion cultivars against damage by severe strains of CTV (Muller & Costa, 1987). Such control measure has also been tried in BBTV in Taiwan. Presently, we are evaluating the effectiveness of using the local symptomless variants in protecting the symptomatic variants.

Conclusion

Recently, international movement of *in vitro* banana plantlets and citrus budwoods for commercial production or germplasm conservation have increased. There is a real and urgent need for the rapid indexing of viruses in banana and citrus. Today, reliable and fast diagnostic methods like ELISA, dot blot hybridization, and PCR amplification are available for detecting these pathogens. These tools, together with sufficient research information on these pathogens for the formulation of quarantine protocols, can now guarantee the safe movement of *Musa* and Citrus species.

In this context, international organizations like FFTC, INIBAP, and IPGRI have played important roles in disseminating information, standardizing quarantine protocols, and supporting meetings, workshops, and training courses.

When discussing virus and virus-like diseases of banana and citrus, I feel there is a dearth of information regarding the insect vectors, especially the ecology of the vectors, their molecular biology, and their natural enemies. One factor that have contributed to the paucity is that, by and large, they are not considered to be important pests on fruit crops when compared to fruit flies, and stem and fruit borers. Efforts should be made to promote their

studies because the availability of this knowledge is crucial in our attempt to formulate an effective integrated management of these pathogens.

References

Allen, R.N. Epidemiological factors influencing the success of roguing for the control of bunchy top disease of bananas in New South Wales. Australian Journal of Agriculture Research 29, 1978. pp. 534-544.

Eng, L. Banana bunchy top virus disease in Sarawak. In: Proc. of the 33rd Annual Research Officers Conference, Dept. of Agric., Kuching, Sarawak, Malaysia, 1996. pp. 163-172.

Garnier, M.; Bove, J.M. Report on virus and virus-like disease of citrus in Malaysia with special reference to citrus greening, 1989. 17p.

Lassoudiere, A. La mosaique dite "a tirets" du bananier Poyo en Cote d'Ivoire. Fruits 29, 1974. pp. 349-357.

Lim, W.H.; Shamsuddin, O.M.; Ko, W.W. Citrus greening disease in Malaysia: Status Report. In: Proc. 4th Asian Pacific Conference on Citrus Rehabilitation. Chiang Mai, Thailand, 1989. pp. 100-105.

Maitree, P. Simultaneous infection of mandarin (*Citrus reticulata* Bl.) with tristeza virus and greening organism in Thailand. In: Proceedings of the 4th International Asia Pacific Conference on Citrus Rehabilitation. Chiang Mai, Thailand, 4-10 Feb 1990.

Matsumoto, T.; Wang, M.C.; Su, H.J. Studies on Likubin. In: Proc. 2nd Conf. IOCV, Univ. Florida Press, Gainesville, 1961. pp. 121-125.

Mink, G.I. Control of plant disease using disease-free stocks. Handbook of Pest Management in Agriculture. Vol. 1. CRC Press, Florida, 1981. pp. 317-346.

Muller, G.; Costa, S.A. Search for outstanding plants in tristeza-infected citrus orchards: The best approach to control the disease by preimmunization. Phytophylactica 19, 1987. pp. 197-198.

Ong, C.A. Studies of cucumber mosaic virus in West Malaysia. B. Agric. Sc. Theses, Faculty of Agriculture, University of Malaya, 1971. 76p.

Ong, C.A. Insect-borne virus and virus-like diseases of tropical fruits in Malaysia. Paper presented in International Symposium on Integrated Management of Insect-borne Virus Diseases of Tropical Fruits. Pingtung, Taiwan, ROC, 1995.

Ong, C.A.; Eng, L.; Su, H.J. Asymptomatic and symptomatic variants of banana bunchy top virus in Malaysia. Paper presented in International Conference on Tropical Fruits. Kuala Lumpur, Malaysia, 1996.

Ong, C.A.; Doon, Y. Viral disease of Malaysian fruit trees. Paper presented in SASS-MAPPS Seminar on Tropical Fruits —Cultivation and Pest& Disease Management. Kota Kinabalu, Sabah, Malaysia, 1988. 12p.

Ong, C.A.; Ting W.P. A review of plant viral diseases in Peninsular Malaysia. Tropical Agriculture Research Series No. 10, 1977. pp. 155-164.

Stover, R.H. Banana, Plantain, Abaca Diseases. Kew, England: Commonwealth Mycology Institute, 1972.

Su, H.J. Tristeza virus strain causing dwarfing of pummelo and grapefruit. In: Proc. Intern. Soc. Citriculture, 1981.

Su, H.J.; Hong, T.H.; Wu, M.L. Preparation and application of diagnostic DNA probe on the fastidious bacteria causing citrus greening. In: Proc. of the Symposium on Research and Development of Citrus in taiwan, 1995. pp. 167-176.

Tanaka, H; Ueno, I. Report on the visit to Malaysia to survey agronomic practices and diseases of citrus, 1978. 22p.

Teo, C.H. Citrus greening and tristeza virus diseases in Sarawak, East Malaysia. Paper presented in International Symposium on Integrated Management of Insect-borne Viral Diseases of Tropical Fruits. Pingtung, Taiwan, ROC, 1995.

Ting, W.P. Tristeza disease of citrus in the Federation of Malaya, F.A.O. Plant Protection Bulletin 10, 1962. pp. 81-83.

Tsai, M.C.; Su, H.J. Development and characterization of monoclonal antibodies to citrus tristeza virus (CTV) strains in Taiwan. In: 11th Conf. CV. IOCV Riverside, 1991. pp.46-50.

Wu, M.L.; Hung, T.H.; Su, H.J. Strain differentiation of cucumber mosaic virus associated with banana mosaic disease in Taiwan. Ann. Phytopathol. Soc. Japan 63, 1997. pp. 176-178.

Session II

Disease Management of Citrus Orchards Planted with Disease-free Seedlings in Thailand

Suchat Vichitrananda
Department of Agriculture
Ministry of Agriculture and Cooperatives
Thailand

Abstract

Under the collaboration between DOA-DOAE and Thai-German IPM in Selected Fruit Tree Project, the Nursery Production Program initiated the production of disease-free Citrus in Thailand. National Citrus Foundation Block was established under the management of DOA and certified budwoods were released to the Approved Private Nursery under the Nursery Accreditation Scheme. Disease-free citrus cultivars budded on Phytophthora tolerant rootstocks i.e., Troyer, Carrizo, and Volkameriana are commercially propagated and released to major growing areas of the country. It is highly recommended that disease-free citrus should be cultivated in an isolated area at least 5 km. away from disease infected citrus orchards. Replanting disease-free citrus trees in established orchard is prone to be re-infested by the disease within 1-2 years. Study on the management to prevent re-infestation of greening and tristeza showed that chemical sprays at 7 day-intervals in the first year could prevent invasion of insect vectors and reduce the risk of disease occurrence.

Introduction

Among fruit species grown in Thailand, citrus is one of the most important fruit commodities with potential for local consumption, export, and import substitution. Citrus (*Citrus* spp.) can be grown throughout the county from lowland areas of the Central and the South to highland areas of the North and Northeastern. The species and varieties that are commercially cultivated consist of Som-kiew-wan (tangerines, *Citrus reticulata*), Som-tra (sweet orange, *C. sinensis*), Som-O (pummelos, *C. maxima*) and Ma-nao (small acid seedy limes, *C. aurantifolia*). As in many Southeast Asean countries, citrus cultivation is mostly family business with farm size ranging between 1-4 ha. Larger farm of 50-1,000 ha has also been successfully established particularly in the North. Among these citrus species, Som-kiew-wan is supposed to be the most popular variety for Thai consumers while Som-O has high potential for export. Som-tra and some other sweet oranges are of minor importance and are grown in small areas in the Central. Ma-nao is widely grown throughout the country in backyard or small farm enterprises. It is the most important as it is used for cooking purposes. In 1989, the planting area of Som-kiew-wan was about 49,730 ha with total production of 702,903 t. The planting area decreased until in 1994 there were only 35,443

ha accounting to 584,245 t of fruits. In contrast, the planting area of Som-O gradually increased from 14,020 ha in 1989 to 20,858 ha in 1994. Consequently, production increased from 70,476 t to 81,558 t. The decrease in planting area of Som-kiew-wan is probably due to severe flooding in the Central and declining tress population caused by certain devastating diseases, i. e., greening, tristeza and Phytophthora root rot (Table 1).

Table 1. Planting areas and production of Som-kiew-wan and Som-O in Thailand for the period 1989-1994.

Year	Som-kiew-wan			Som-O		
	Planting area (ha)	Production area (ha)	Yield (t)	Planting area (ha)	Production area (ha)	Yield (t)
1989	49,730	35,606	702,903	14,020	9,599	70,476
1990	48,392	36,136	656,668	14,915	9,957	69,801
1991	47,294	27,011	469,770	14,505	9,890	67,850
1992	40,269	33,011	680,971	14,496	9,374	75,637
1993	38,255	30,063	609,753	15,506	10,029	81,372
1994	35,443	27,529	584,245	20,858	10,344	81,558

Source: DOAE

Greening disease is considered the most serious disease of tangerine. It is a vascular disease caused by bacteria-like organisms that can be transmitted by vegetative propagation, grafting, budding or marcotting; and by insect vector, psyllids-*Diaphorina citri.* The disease is known as yellow shoot (Huanglungbin) in China, decline (likubin) in Taiwan, dieback in India, leaf mottle in the Philippines, vein phloem degeneration in Indonesia and yellow branch, blotchy-mottle or greening in South Africa. Schwarz et al., reported that greening disease first appeared in Thailand in the 1960s. The disease has now spread all over the country and serious decline of tree population was observed in many growing areas. The disease destroys about 10-15% of the total tangerine population each year in Thailand and is exceptionally destructive in the North.

Tristeza is a viral disease that can cause great damage to certain stock-scion combination and even of some varieties when grown as seedlings. Symptoms include bronzing of leaves, leaf yellowing, foliage wilts, leaf drops, twig and root dieback, and stem pitting or honey combing on wood. The disease can be transmitted by means of vegetative propagation and insect vectors such as black citrus aphid (*Toxoptera citricidus*) and cotton aphids (*Aphis gossipii*). Tristeza disease is also widespread sometimes occurring with greening disease in citrus species grown throughout the country. Among citrus species, ma-nao (*C. aurantifolia*), is more susceptible to tristeza virus. Infected trees show yellowing of the leaves, vein clearing and corky vein of the leaves and also stunting of the tree.

Root and stem rot caused by *Phytophthora parasitica* is also a serious disease of citrus especially those grown in lowland areas. Som-kiew-wan is more susceptible to Phytophthora rot. The fungus can attack roots and bark of the stem resulting to discoloration of bark tissues and rotting of fibrous roots or sometimes, the whole root system.

Among these diseases, greening and tristeza are the main causes of death in Som-kiew-wan trees in Thailand. Due to the technique of propagation by marcotting, these two vascular diseases can widely infest every citrus planting areas. It is believed that most of citrus trees grown in the country are infected with at least one of the two vascular diseases. Field investigations

on greening disease and its vector *D. citri* have been carried out in 12 district throughout Thailand from 1992-1993 by Koisumi *et al*. These confirmed the presence of the disease and its vector in all citrus orchards surveyed. The disease primarily affects yield and life span of citrus tree. Tree growth and yields are markedly reduced when trees are infected at very young ages. The average yield of Som-kiew-wan at 13 t/ha is low when compared to yields of 50-90t/ha in other countries. Control of greening and tristeza can be achieved by integrated measures such as eradicating infected materials, introducing diseased-free planting materials and eliminating insect vectors. Studies in the Philippines, South Africa and China indicated that greening disease can be successfully controlled. All of these disease control programs followed a system of management which include: 1. planting of disease-free, certified nursery stock; 2. monitoring the presence of psyllid vector and the timely application of pesticides to control psyllids; and, 3. removal of greening-infected tree and all symptomatic plant tissue.

Disease Management and Citrus Rehabilitation Program in Thailand

In Thailand, the major causes of yield loss and decline of citrus trees are greening, tristeza and Phytophthora rot disease. In the past 20 years, the combination of these diseases could destroy almost all citrus trees in large citrus growing areas such as Petchaboon, Chantaburi, Trad, and Rayong Provinces. At present, there are only few old citrus orchards left. Newly established orchards are planted only with healthy marcotted/budded rootstock. Nowadays, citrus decline is common to all old established orchards in Pathumthani, Phrae, and Nan Provinces which were used to be the largest and most important citrus-growing areas of the country. Prathmthani and nearby provinces in Central Thailand are the largest planting areas for Som-kiew-wan accounting to about 75% of all citrus planting areas. Citrus in lowland area are grown on raised bed surrounded with water ditches. This differs from the citrus cultivation in plain or lowland areas in northern provinces. In lowland areas, the growers can initiate flower induction by controlling the amount of water in the ditches and fertilizer application. Due to the continuous production of fruits and the problem of greening or tristeza disease, citrus trees in these areas usually have short lifespan. In some orchards, 4-5 year-old trees suddenly die or complete only about 2 or 3 fruiting cycles. In general, the average lifespan of citrus in these areas is about 8-10 years with the productive period of 5-7 years. In citrus orchards, the death of 1-2 year old trees due to disease is common. In order to control pests and diseases, particularly in the areas where citrus are extensively grown, the growers have to use a lot of pesticides. Fosethly-Al and metalaxyl are chemicals that are widely used for the control of Phytophthora rot in citrus. The success of the control measures depends on the disease severity and the environmental conditions that are favorable to disease outbreaks. However, until now there is no chemical available for the control of greening and tristeza diseases.

In an attempt to solve the problem of citrus decline caused by greening and tristeza diseases, the disease-free Citrus Nursery Program was implemented by Thai-German IPM Project with the cooperation of

the Department of Agriculture (DOA) and the Department of Agricultural Extension (DOAE) in 1993. In the beginning of the program, several techniques were tested to obtain disease-free plants of different citrus varieties and cultivars. The techniques found to be reliable and efficient were heat treatment of infected citrus plants with specific temperatures, shoot tip grafting, and early detection of the presence of greening organisms and viruses in plant tissues. By using combined techniques of chemotherapy and shoot tip grafting, disease-free foundation stock trees were produced for mass propagation. At present, there are available disease-free mother trees of many citrus varieties and cultivars in Thailand. The disease-free mother trees are being kept in the insect-proof screenhouses at Bangkhen area in Bangkok under the management of DOA Nursery Team. From the disease-free mother trees, used as budwood source, disease-free plants could be produced by budding clean budwoods on selected rootstocks.

Normally, Thai citrus growers use marcotting or air layering to propagate citrus trees. However, they have been taught that propagation by using marcotting or even grafting or budding from disease-infected trees may enhance the spread of greening and tristeza disease. In Som-kiew-wan and other tangerines, the disadvantage of using marcotted is planting materials that they are more susceptible to Phytophthora root rot. The use of disease-free citrus on resistant rootstocks may help in solving the problem of Phytophthora infection. In his report, Tolley recommended *Poncirus trifoliata,* Troyer, and Carizzo Citrange as rootstocks for Som-kiew-wan and Som-O.

Production and Distribution of Disease-free Plants

The National Citrus Foundation Block was established in 1994 at Bangkhen area to maintain the registered disease-free mother trees. DOA is responsible for the maintenance of the National Foundation Block and Certified Mother Tree Block. Disease-free budwoods are produced from Certified Mother Trees and released to the Approved Citrus Nurseries, Government Agencies or Farmer Groups. In order to improve the quality of nursery produce, the Nursery Accreditation Scheme was initiated in 1993. The nursery owners who want to produce disease-free citrus plants for commercial purposes must follow the conditions, regulations and recommendations indicated in the scheme. Certificate of Accreditation, issued for 12 months, are provided to nurseries that have the approved insect proof screenhouses and produced disease-free plants that meet the quality standards. The Certified Citrus Nursery are inspected at least twice a year for the renewal of the certificate. At present, there are many certified citrus nurseries in the country, two of which were producing Certified Citrus Nursery disease- free plants on a commercial scale. For the past two years (1996-1997), at least 1,501,408 disease-free citrus plants were produced by the two nurseries. The plantlets produced were distributed to citrus growing areas for replanting or establishment of new orchards (Table 2). Some disease-free plants were grown in new areas that are isolated from old established orchards such as in the northeastern part of the country.

Table 2. Number of disease-free citrus plants produced and distributed by the two leading Certified Disease-free Citrus Nurseries in 1996-1997.

Citrus cultivars	Number of disease-free plants distributed	
	1996	1997
Som-kiew-wan	443,962+	498,750+
Som-Sho-kun	4,310+	4,420+
Som-O	359,010+	290,271+
Fremont		

Source: Nursery's Record

Growers' Acceptance

There is a common misconception about disease-free citrus plants. Most farmers believe that disease-free citrus plant are resistant to pathogens when grown in orchards. This is definitely not true. Also, citrus growers in lowland areas also believe that high level of water may affect the root system of rootstocks and citrus budded on rootstocks might have difficulties in inducing off-season fruits. However, through proper education, many citrus growers are now more interested in replanting disease-free budded rootstocks in old unproductive orchards or planting them in new areas. The use and cultivation of disease-free citrus trees in Thailand could be described by the following:

a) Use of disease-free citrus plants to replace dead trees in orchards. This practice is usually done by some growers in Rangsit area, Prathumthani Province.
b) Grow the disease-free citrus plants in plots surrounded with citrus trees infected with greening and tristeza disease. This practice is observed in many areas in Prathumthani, Phrae, and Nan Provinces.
c) Disease-free citrus plants are grown in new areas far from old citrus orchards. New areas for citrus growing should be in the northeastern part of the country where field crops and rice are the main crops. Many areas in this part of the country are found to be far from citrus orchards and probably suitable in growing disease-free citrus plants. Citrus growing is suitable in Udornthani, Loei, Srisaker, and Buriram.

Apart from this, some growers consider healthy-looking citrus trees but severely infected with greening disease good source for propagation purposes. Growers take buds from these trees and graft or bud them on rootstocks such as Cleopatra mandarin, Troyer Citrange, and Swingle Citrumelo. These greening or tristeza-infected trees may grow very well in the beginning and could tolerate the infection of Phytophthora disease. However, some of these trees may show deficiency symptoms 3-4 years after fruit production due to the development of the disease inside plant tissues. The practice of using disease-contaminated tissues for propagation may delay or obstruct the development of the citrus Rehabilitation Program initiated by DOA . In the long run, these healthy-looking trees will become the source of inoculum for the recontamination of the disease-free citrus trees. Growing disease-free citrus in isolated areas is supposed to be the best way to keep the trees free from greening and virus disease infection if vectors are successfully controlled. Nevertheless, growing disease-free citrus among the disease-infected trees or replanting them near established orchards could not be avoided in the present situation.

Management of Disease-free Citrus Orchard

The general recommendations for management of citrus greening disease are as follows:

1. In isolated new citrus-growing areas or at least 5 km from citrus orchards, the use of disease-free citrus cultivar is highly recommended.
2. Remove possible sources of infection around newly planted orchards or around established orchards such as old non-productive trees. Citrus trees grown in backyard and abandoned orchards in the area should also be removed.
3. Remove all poor performing trees and replaced them with disease-free trees.
4. During cultural operation, avoid damaging bark and root system.
5. Establish windbreaks around the orchard.
6. If possible, synchronize flushing.
7. Monitor pests and diseases during each new flush. Check each new flush for eggs, nymphs, and adults of citrus psyllids. Check on about 5% of tree in the orchard for the presence and absence of psyllids.
8. Economic Threshold Level (ETL) should be about 10% shoots with more than 1 egg, nymph, or adult.
9. When the ETL is reached use a jet spray of water to wash down the eggs and nymphs and monitor the development of the population.
10. When the ETL is reached, pesticide sprays for psyllid control may be applied, following the IPM recommendations. The chemical should be selected according to the target pest or pests. During new flushes, it is likely that aphids, leaf minor, and thrips may also be found above the ETL.
11. In the first 2-3 years of plant development, insect vectors must be completely eliminated.
12. Branches or twigs that show symptoms of greening disease must be removed and destroyed. Cut end should be treated with fungicides such as copper oxychloride.

In the initial phase of the greening and tristeza disease control program, disease-free Som-kiew-wan or Rangpur Lime rootstocks were planted in demonstration plot about 0.3 ha in size at the Chiangrai Horticultural Center. This plot was located near plots of Som-kiew-wan, Freemont, and Som-O grown through marcotting and infected with greening or tristeza diseases. The disease-free plants grew very well during the first 3 years after planting and produced quality fruits. Due to poor pest management practice, some trees showed greening and tristeza symptoms in the fourth year. It was observed that the severely disease infected trees were usually found in the front row adjacent to the disease-infected plot.

Another trial was made in the same area, using disease-free Som-kiew-wan on different rootstocks i.e., *Poncirus trifoliata,* Troyer, Rough Lemon, and Volkameriana. In the first year, spray program was set to control insects and canker disease. Insecticides such as methamidophos, carbosulfan, imidaclopid, and methomil were alternately sprayed at 7-day intervals to control leaf-miners, thrips, aphids, and psyllids. To control the disease, particularly canker, copper oxychoride was mixed with insecticides and sprayed during the flushing period. One year after planting, leaf samples

were taken from every tree. The leaf samples were subjected to ELISA test to determine the presence of tristeza virus. The results showed none of the tree was infected with tristeza virus. On the second year, the intervals of spray program was changed to ten days. Observations made at nearly two years after planting found damages on some leaves caused by the infestation of aphids. The presence of tristeza virus are checked when trees are already two years of age.

Another experiment evaluated the effect of certain rootstocks on Som-kiew-wan growth and production in Pathumthani. Results showed no difference in the growth of the scion using different rootstocks i. e., Troyer, Carizzo, Cleopatria and Volkameriana. The trees are now three years old and bearing fruits. However, due to the proximity of experimental plot to severely infected areas and poor spray program, some trees in the plot were found to be re-contaminated with disease. The trees showed symptoms of greening disease on leaves and twigs. Psyllids and aphids were found on young shoots.

Observations on the performance of disease-free Som-kiew-wan budded on Troyer rootstocks in growers' orchards were made in Pathumthani, Udornthani, Phrae, Nan, and Loei Provinces. The trees in these orchards were 2-3 years old. It was found out that Troyer rootstocks developed faster than Som-kiew-wan scions. Most of the disease-free trees grew well and looked very healthy. Disease management is different among the orchards due to the difference in experience and knowledge of each grower. The chemicals normally used in the orchards are insecticides such as dimethoate, carbosulfan, imidaclopid, and fungicides such as carbendazim, copper oxychloride, and mancozeb. These pesticides were applied to control major citrus pests i.e., leafminers, thrips, and canker. Aphids and psyllids are small insect vectors that must be eradicated immediately. Unfortunately, many growers do not know these insects and their role in infecting disease-free plants. In orchards where spray program for insect control has not been applied, psyllids or aphids usually infested young leaves of the trees. There was also no evidence of Phytophthora infection in all recommended rootstocks both in the experimental plots and private orchards.

References

Buitendag, C.H.; von Broembsen, L.A. Living with citrus greening in South Africa. In: Proc. 12th Conf. IOCV, Riverside, 1993. pp. 269-273.

Gonzales, C.I.; Vinas, C.R. Field performance of citrus varieties and cultivars grown under control measures adapted against leaf mottling (greening) disease in the Philippines. Proc. Int. Soc. Citriculture. 1, 1981. pp. 463-464.

Gonzales, C.I.; Toreja, A.D.; Molino, U.V. Status of citrus plantings on the ninth year under study for control procedures of greening disease in the Philippines. Proc. Int. Soc. Citriculture. 1, 1984. pp. 409-412.

Grenzebach, E. Integrated pest management in selected fruit trees. Report No. 42 on a short term consultancy mission:Feb. 27-March 12, 1994 on behalf of Deuche Gesellschaft Fur Technische Zusammenarbeit (GTZ).

Ke, C.; Xu, C.F. Successful integrated management of Huanglongbing disease in several farms of Guangdong and Fujian by combining early eradication with targeted insecticide spraying. In: Proc. 4th Int. Asia Pacific

Conference on citrus rehabilitation, Chiang Mai, Thailand, 1990. pp 145-148.

Ke, C. The present status of citrus huanglongbing and its control in China. Rehabilitation of citrus industry in the Asia Pacific region. In: Proc. 6th Int. Asia Pacific Workshop Integrated Citrus Health Management, Kuala Lumpur, Malaysia, 1991. pp. 10-14.

Koizumi, M.; Prommintara, M.; Linwattana, G.; Kaisuwan, T. Epidemiological Aspects of Citrus Huanglongbing (Greening) Disease in Thailand. JARQ 31, 1997. pp. 205-211.

Roistacher, C.N. A report on a technical visit to Thailand to assess citrus certification and the production of disease-free citrus. With special emphasis on the greening problem recommendations for rootstocks for Thailand, 1994. 33 p.

Schwarz, R. E.; Knorr, Prommintara, M.; Greening-cause of a recent decline of citrus in Thailand. Plant Protection Service Technical Bulletin No. 20. October, 1973. Department of Agriculture, Ministry of Agriculture Cooperatives, Thailand. 17 pp.

Xu, C.F.; Wang, D.C.; Ke, C. A report on the implementation of integrated control programme of citrus Huanglongbing, aiming at renovating old infected orchard in epidemic zone and protecting new non-infected orchard in non-infected zone. Rehabilitation of citrus industry in the Asia Pacific region. In: Proc. 6th Int. Asia Pacific Workshop on Integrated Citrus Health Management. Kuala Lumpur, Malaysia, 1991. pp. 55-61.

Tolley, I.S. A report and review 3rd mission consultancy to Thailand on citrus nursery orchard production. Report No. 43 Thai German Plant Protection Program (TG-PPP). 48p.

Recent Progress on Citrus Greening Research in Asia Including a Serological Diagnosis

Yoshihiro Ohtsu
National Institute of Fruit Tree Science
Japan

Abstract

Recent studies on citrus greening disease in Asia were reviewed in this paper. The wood apple, *Limonia acidissima*, was found as a new host for the greening vector, *Diaphorina citri* in Thailand. Detection of 16 S rDNA fragments of greening organisms (GO) by polymerase chain reaction (PCR) was used and improved with Thai GO isolates maintained in citrus plants. The 16 S rDNA fragments of GO were detected in one leaf of Thai mandarin and Nepalese mandarin each showing seven types of greening symptoms, as well as in one insect vector *D. citri*. Sequence analysis of the 16 S rDNA fragments and the 16 S / 23 S intergenic spacer regions of Nepalese and Thai isolates of GO showed that these isolates were closely related to Indian and Chinese isolated of *Liberobacter asiaticum*. A serological diagnosis using a microprecipitin test was developed for greening disease. A clear plus reaction was found in a mixture containing diseased citrus extracts and antiserum, but was not found in a mixture with citrus extracts infected with attenuated citrus tristeza virus that may have contained the pathogen-related protein.

Introduction

Citrus greening disease is one of the most destructive citrus diseases in Africa and Asia, including Thailand and the Arabian Peninsula. The pathogen of the disease is a bacterium restricted to the phloem of infected plants and is called a greening organism (GO). The GO has two strains, Asian and the African. These are transmitted by two psyllid vectors, *Diaphorina citri* and *Trioza erytreae*, in Asia and Africa, respectively. From a comparison of the sequences of 16 S rDNAs of GO with those obtained from the Genebank database, the scientific names of *Liberobacter africanum* and *Liberobacter asiaticum* were proposed for the African and Asian isolates of GOs, respectively. The sequences of 16 S / 23 S ribosomal intergenic regions of GOs were compared: the intergenic spacer of *L. asiaticum* contains the genes of isoleucine tRNA ($tRNA^{Ile}$) and alanine tRNa ($tRNA^{Ala}$), and the intergenic spacer of *L. africanum* contains only one tRNA ($tRNA^{Ala}$) gene.

Although a polymerase chain reaction (PCR) diagnosis of the disease has been used, a serological diagnosis is a simple and cheaper method than PCR. The Poona strain of GO from infected periwinkle was purified using the monoclonal antibody MA (10A6) in affinity chromatography. The purified GO preparations were shown to have a filamentous structure of 1-4 μm in

length and 0.15 -0.3 μm in diameter. Round form structures of GO were also observed with a diameter of 1.0 μm. However, production of an antiserum against GO was not reported. An antiserum against an extract of greening-infected citrus material in South Africa was produced, but the antiserum reacted with a disease shock protein common to all the diseases. Polyclonal ascites antiserum produced against a partially purified periwinkle material infected with the huanglungbing strain of GO in Taiwan, but the titer of the antiserum was low and interference from non-specific reaction was obvious.

In this study, recent studies were reviewed on citrus greening disease including serological diagnosis.

A New Host for the Greening Vector

Approximately six-month-old seedlings of 15 Rutaceae plants, including three citrus cultivars, were exposed to *D. citri* that had fed on greening-infected citrus plants collected from Thailand. Long-term survival of the psylla of more than seven weeks was observed on the following plants: *Balsamocitrus dawei, Murraya paniculata, M. koenigii, Limonia acidissima* (wood apple), *Atalantia* sp., *Severinia buxifolia, Poncirus trifoliata,* and Som-pan and Som-keo-wan mandarins. Multiplication of psylla was noted on the nine plants particularly *M. paniculta*, *Atalantia* sp. and *L. acidissima*. The former two developed no symptoms, but *L. acidissima* developed leaf mottling and yellowing. An electron microscope study failed to show conclusive evidence of greening organisms in the sieve cells of infected *L. acidissima*. These results showed that *L. acidissima* is a new host for *D. citri* and needs further investigation as a possible host of the greening agent.

Simplifying the DNA Extraction Procedure for PCR Diagnosis

The PCR method to amplify the 16 S rDNA fragments of the GOs was developed by Jagoueix et al. This method was used to detect Thai GO (Nakorn-Pathum) isolates. By simplifying the DNA extraction procedure, DNA was obtained for the PCR within 20 minutes and detected the GO within 4.5 hours. The Thai isolates were shown to be phylogenetically close to an Indian isolate of *Liberobacter*.

Detection of GO in Citrus Plants and in the Psylla *D. citri* in Thailand

The PCR method was used to detect 16 S rDNA fragments of GOs in leaves with seven kinds of symptoms collected from GO-infected citrus trees in Thailand. The symptoms were mottling (Type I), mild chlorosis with green veins (Type II), severe chlorosis with green veins (Type III), pale green color in young leaves (Type IV), vein yellowing (Type V), vein corking (Type VI) and unclear symptoms (Type VII). The GO DNA was high in leaves with Types I,II,III,V, and VI symptoms. GO DNA was also detected in the insect vector *D. citri*. Sequence analyses of the amplified 16 S rDNA fragments of seven Thai isolates collected from six major citrus-producing areas showed that the sequences of the 16 S rDNA fragments of these isolates were the same and very similar to that of the Indian isolates of *L. asiaticum.*

Typical Symptoms of Greening on Mandarin Trees Supported by Detecting GO DNA in Nepal

Citrus greening disease, caused by greening organisms (GOs; *Liberobacter* spp.), is also a destructive disease of citrus in Nepal and Thailand. The 16 S rDNA fragments of GOs were detected by PCR in leaves showing one of the seven typical symptoms: mottling (Type I), chlorosis with green net like veins (Type II), severe chlorosis with green main veins (Type III), pale green on young leaves (Type IV), vein yellowing (Type V), vein corking (Type VI), and yellow blotching (Type VII). Leaves were collected from GO-infected 'Suntala' mandarin trees in Nepal. Sequence analysis of the 16 S rDNA fragments and the 16S/23 S intergenic spacer regions of Nepalese and Thai isolates of GOs showed that these isolates were closely related to Indian and Chinese isolates of *L. asiaticum*. Symptom types III, V, and VI were considered to be optimum for PCR diagnosis because of the high amount of GO DNA amplified. The accuracy of diagnosis of greening by the naked eye can be improved by observing as many leaves as possible with all seven typical symptoms on the same mandarin tree. Symptom types III, V, and VII are common to mandarin and sweet orange because these symptoms are similar to those reported on sweet orange in South Africa.

Periwinkle Plants Symptoms Expression and Presence of Pathogen in Greening-Disease

Periwinkle (*Catharanthus roseus* L.) is a suitable plant to propagate GO. Four-month-old periwinkle plants were graft-inoculated with GO-infected buds, and were maintained in a greenhouse at 24-27°C until they were placed in the chambers to be tested. The temperatures under warm conditions in the chamber were 30°C for 12 hours during the day and 25°c for 12 hours at night. Temperatures under cool conditions were 25°C for 12 hours during the day and 20 °C for 12 hours at night.

The symptoms appeared on one or two leaves of new shoots immediately below the graft insertion at 26-35 days after graft-inoculation, and on all leaves of the lower new shoots 29-41 days after graft-inoculation. Yellowing symptoms appeared on mature leaves below the graft insertion 4.5-15 days after the temperature treatment (34-39 days after graft-inoculation). At 20-25°C, the number of leaves showing yellowing symptoms was 4-6 times more than at 25-30°C.

Greening organisms were detected in the sieve tubes of the periwinkle plant with yellowing symptoms. The yellowing mature leaves at 20-25°C were confirmed to have sieve tubes with many GO.

As a conclusion, the following process used to treat the plants is very useful to obtain many leaves with a high concentration of GO. The inoculated periwinkle plants are placed at 25-30°C for four weeks, and then placed at 20-25°C for 1-2 weeks. Infected leaves with a high concentration of GO were obtained from these infected periwinkle plants by collecting mature leaves showing yellowing symptoms and young leaves showing yellowing symptoms.

Partial Purification and Antiserum Production of Thai Isolate of GO

Sieve tissue of periwinkle was prepared with a little modification as reported by Lee and Davis. Midribs (10g) were separated with sharp forceps from Thai-GO-infected periwinkle leaves showing symptoms, and their surfaces were sterilized. Phloem tissues were separated after digesting the diseased midribs in an enzyme solution of 100 ml sterile distilled water, 0.8 g cellulose, 0.4 g macerozyme, 1.0 mM $CaCl_2$-H_2O, 0.5 g PVP-40, and 12.5 g mannitol at room temperature (24-31°C) overnight. The phloem tissues were washed five times in extraction solution (0.6 M mannitol, 25 mM Tris-HCI and 5 mM magnesium acetate, pH 7.5 with little modification as reported by Nakashima and Hayashi), and were macerated in 5 ml of the extraction solution. The pellet was resuspended in 1 ml of the extraction solution after one cycle of centrifugation (29 x g, 5 minutes and 17,000 x g,, 30 minutes of the homogenate. The 16 S rDNA of GO were detected by PCR using the suspension. The suspension was treated with 4% glutaraldehyde in the extraction solution by dialysis for one night, and then was dialyzed in the extraction solution for one day and night. The supernatant was carefully collected after centrifuging at a low speed using a 20% sucrose cushion, and was centrifuged on 20% sucrose cushion at high speed. The pellet was resuspended in 500-1,000 μ1 of the 0.85% NaCl solution.

An emulsion of the suspension and Freund's incomplete adjuvant was injected five times into rabbits. The antiserum had a homologous titer of 1/16 with partially purified GO from diseased periwinkle extracts in a microprecipitin test. A clear serological diagnosis of GO-infected citrus was developed using the microprecipitin test. A clear plus reaction was found in a mixture containing diseased citrus extracts and antiserum, but was not found in a mixture with citrus extracts infected with attenuated citrus tristeza virus that may have contained the pathogen-related protein.

References

Aubert, B.; Garnier, M.; Cassin, J.; Bertin, Y. Citrus greening disease survey in Eastern and Western African countries south of Sahara. In Proceedings of 10th conference of International Organization of Citrus Viroligists, 1988. pp. 31-33.

Bove, J.M.; Calavan, E.C.; Capoor, S. P.; Cortez, R.E.; Schwarz, R.E. Influence of temperature on symptoms of California stubborn, South African greening, Indian citrus decline and Philippines leaf mottling disease. In: Proc. of 6th conference of Internation Organization of Citrus Virologists, 1974. pp.12-15.

Bove, J.M.; Garnier, M. Citrus greening and psylla vectors of the disease in the Arabic peninsula. In Proceedings of 9th conference of International Organization of Citrus Virologists, 1983. pp. 109-144.

Jagoueix, S.; Bove, J.M.; Garnier, M. The Phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the proteobacteria. Int. J. Sys. Bacteriol, 1994. pp. 377-386.

Jagoueix, S.; Bove, J.M.; Garnier, M. Comparison of the 16 S/23 S ribosomal intergenic regions of *"Candidatus Liberobacter asiaticum"* and *"Candidatus Liberobacter africanum,"* the two species associated with citrus Huanglongbing (greening) disease. Int. J. Sys. Bacteriol. 47, 1997. pp.224-227.

Ke, C. et al. Purification and serology of the organism associated with citrus Huanglungbin. In: Proc. of 12th conference of Internationa Organization of Citrus Viroligists, 1933. pp. 220-223.

Koizumi, M.; Prommintara.; Ohtsu, Y. Wood apple, *Limonia aciddima*: A new host for the Huanglongbing (Greening) vector, *Diaphorina citri*. In: Proc. of 13th conference of International Organization of Citrus Virologists, 1996. pp. 271-275.

Lee, I.M.; Davis, R.E. Phloem-limited prokaryotes in sieve elements isolated by enzyme treatment of diseased plant tissues. Phytopathology 73, 1983. pp. 1540-1543.

Nakashima, K.; Hayashi, T. Multiplication and distribution of rice yellow dwarf phytoplasma in infected tissues of rice and green rice leafhopper *Nephotettix cincticeps*. Annals of the Phytopath. Phytopathol. Soc. Jpn. 61, 1995. pp. 451-455.

Nakashima, K.; Ohtsu, Y.; Prommintara, M. Detection of Citrus Greening Organism in citrus plants and Pyslla *Diaphorina citri* in Thailand. Annals of the Phytopath. Phytopathol. Soc. Jpn. 64, 1998. pp. 153-159.

Nakashima, K.; Prommintara, M.; Ohtsu, Y.; Kano, T.; Imada, J.; Koizumi, M. Detection of 16 S rDNA of Thai isolates of bacterium-like organisms associated with greening disease of citrus. JIRCAS Journal 3, 1996. pp. 1-8.

Ohtsu, Y. Present situation and problems of studies on putative pathogen of Citrus Greening Disease. Shokubutsu boeki (Plant Protection) 50, 1996. pp. 236-239.

Ohtsu, Y.; Nakashima, K.; Prommintara, M.; Tomiyasu, Y. Typical symptoms of Citrus Greening on mandarin trees, supported by detection and characterization of ribosomal DNA of the causal organisms in Nepal. Ann. Phytopathol. Soc. Jpn. 64 (6), 1998. (in press).

Ohtsu, Y.; Prommintara, M.; Goto, T.; Okuda, S.; Imada, J. Symptoms expression and presence of pathogen in periwinkle plants graft-inoculated with Thai isolate of bacterium-like organisms (GO) associated with greening disease of citrus. Ann. Phytopathol. Soc. Jpn. 61 (6), 1995. pp. 609.

Ohtsu, Y.; Prommintara, M.; Okuda, S.; Nakashima, K.; Koizumi, M.; Choopanya, D. Partial purification and antiserum production of Thai isolate of bacterium-like organisms (GO) associated with greening disease of citrus. Ann. Phytopathol. Soc. Jpn. 61 (6), 1995. pp. 610.

Villechanoux, S.; Garnier, M.; Bove, J.M. Purification of the bacterium-like organism associated with greening disease of citrus by immunoaffinity chromatography and monoclonal antibodies. Current Microbiology. 2‘, 1990. pp. 175-180.

Whitlock, V.H.; Chippindall, R.J. Greening disease in South Africa — A Multi-Pathogen Syndrome. In Proceedings of 12th conference of Internation Organization of Citrus Virologists, 1993. pp. 233-243.

Ecology of the Insect Vectors of Citrus Systemic Diseases and Their Control in Taiwan

Chiou-nan Chen
National Taiwan University

Abstract

The ecology of the Asiatic citrus psylla (*Diaphorina citri*) in relation to the epidemics of citrus huanglungbin (greening) with a note on the citrus brown aphid (*Toxoptera citricidus*) and the control measures of both vectors are briefly reviewed in this paper. The female psylla lays eggs exclusively on the new shoots of its host plants, especially active during spring flushes. They lay eggs repeatedly whenever new shoots are available. The generation time varies from 18-60 days in different seasons. It completes 8-10 generations a year in Taiwan. The adults and 4-5th instar nymphs can acquire the pathogen from infected plants after feeding for 30 minutes or longer. The latent period is 3-26 days. The pathogen is only spread by the adult. Inoculation feeding takes about 1 hour or longer. The transmission is persistent throughout the adult life span (ca 3-4 months or longer). However, the virulence cannot be transferred to the progeny via eggs. Healthy Ponkan trees could be infected five months after planting, with 89% infection three months later, as detected by DNA probing. Besides control technology, the importance of the integration of manpower, financial support, and law enforcement is also emphasized in the implementation of the control program.

Introduction

Citrus huanglungbin (likubin, greening) and tristeza have been important systemic diseases devastating citrus production in Taiwan since the 1950s. Both pathogens could be transmitted by grafting and propagating with infected plant material as well as by insect vector. It is essential to have a sound knowledge about the epidemics of these diseases so as to design an integrated control program. The coupling of three components in a pathosystem is required for the completion of a disease cycle and its spread. In this regard, we need to understand the behavior of the pathosystem in terms of interactions among the causal organism, the host plant and the vector under prevailing conditions. Theoretically, a destruction of any linkage between two components would disrupt the coupling of the pathosystem and bring it into collapse – the aim of an effective control of the disease. Several approaches have been taken in order to reach this aim. These include the destruction and elimination of the sources of inoculum, the control of insect vectors and replanting with pathogen-free healthy seedlings. In any respect, complicated technology and know-how are involved and they

should be integrated in a concerted way in order to do the job. In spite of that, the paper will focus mainly on the ecology and control of insect vectors involved.

Ecology of the Insect Vectors

A. The Asiatic Citrus Psylla (*Diaphorina citri* Kuwayama)

The psyllid is the vector of the citrus huanglungbin (HLB) pathogen, a fastidious, gram-negative bacterium, inhabiting the phloem of the citrus plant. It mainly infests Rutaceae plants especially citrus and jasmine orange (*Murraya paniculata* (L.) Jack). This psylla has been found in the hot and dry areas of Southern mainland China, Taiwan, southern islands of Japan, the Indian subcontinent, the western part of the Arabian Peninsula, Southeast Asia, Reunion Island, Mauritius, and Brazil where citrus is produced.

Three Natural enemies are associated with the psylla in Taiwan, including two predators (*Chrysopa boninensis* and *Menochilus sexmaculatus*) and an endoparasitoid (*Diaphorencyrtus diaphorinae*). A nymphal ectoparasitoid, *Tamarixia radiata* was introduced and established in the mid-1980s to enhance natural regulation of the psylla population on jasmine orange. In addition, 11 hyperparasitoids attacking the parasitoids are also present in the life system of the citrus psylla in Taiwan.

1. Life History and Habits

Female psylla lays eggs exclusively on new shoots of the host plants. During her lifespan of 3-4 months, a female could repeatedly lay eggs, of up to 200-800 eggs during an oviposition period of about 30-80 days. At 25° C, the egg incubation period is four days. The nymphs aggregate underneath the leaf. They suck phloem sap from succulent leaves or petioles and excrete white pellets or threads, covering the shoots and dusting the lower leaves and inducing sooty mold growth. It takes 15 days to complete the five nymphal instars. The preoviposition period is from 17-60 days.

The generation time is temperature-dependent. Xu *et al.* reported that it takes 53-56 days in the spring (March to May, mean 10-day temperature 28° C), and 25-30 days in the autumn (September to November, mean 10-day temperature 24° C) to complete a generation.

In general, the psylla could complete 8-9 generations annually in the northern part of Taiwan, probably ten or more in southern Taiwan, 11-14 in Guangdong, 6-7 (on citrus), and 9-11 (on Murraya) in Fujian, and 6-7 in southern Zhejiang. The favorable temperature is 22-29° C, below 20° C the psyllid population declines significantly, probably because of lack of new shoots. The lethal temperature of nymph is –3° C for 1 hr, and adult –10° C. Exposure under –8° C for six days causes 91% mortality; while –10° C for three days, 55% and for five days, 89% mortality.

2. Transmission of the HLB Bacteria

Citrus psylla can transmit the HLB pathogen despite very low efficiency (Ca. 1%). The 4-5th instar nymphs and adults are able to acquire pathogens after feeding on infected plant for 30 minutes or longer. The pathogens remain latent inside the vector for about 3-20 days before being detected in the salivary gland. The inoculation feeding takes about 1 hour or longer. Once the psyllid vectors acquire the bacteria it could transmit the pathogens throughout their lifespan. However, it could not transfer the virulence to its progeny via eggs. The latent period in citrus plant ranges from four months to one year or more before symptoms are expressed. The infected young plants would die in 2-4 years.

3. Ecology of the Vector in Relation to Epidemics of the Disease

The adult citrus psylla occurs on citrus and Murraya plants all year round. They will continue to lay eggs on the plant throughout the year whenever new flushes are available. Population density of eggs and nymphs is entirely dependent on the availability and abundance of new plant shoots, which could be triggered by typhoon damage or by pruning. Irregular psylla population fluctuation was observed corresponding to the flushing of new shoots. Under natural conditions, citrus flushes three times a year – during spring, summer, and autumn. The abundance decreases in that order. Thus, psylla population builds up quickly from mid-March and reaches its peak in late April to early May. It then declines in July and in early November. Yellow sticky traps catch the highest number of adults during March to May, indicating the most active egg-laying taking place in the spring flushes. This is considered to be the most important time for chemical application.

The transmission rate of HLB is high in March, April, and May (40, 33, and 20% respectively) based on a monthly infection test. Under field conditions with diseased plants and insect vectors present, a plot with 30 healthy plants was 57% infected in six months, a year later 73% and 100% infected two years later as detected by indexing. In fact, the dispersal of the psylla in the orchard is rather slow. In a plot (30m X 15m) planted to 60 seedlings during February, it took about two months after release for the psylla to spread all over the plot.

Wang *et al*. conducted an in-depth study of the disease epidemics by using dot hybridization test with DNA probe for detecting HLB organism in psylla and plant. In a 0.78 ha plot, 240 healthy Ponkan seedlings (1.8 X 1.8 m spacing) were planted in November 1991. Invasion of psylla on plants was observed five months later (April 25, 1992). The infestation rate was 8%. Monthly survey showed that it increased to 23% in May and 73% in July. DNA probing in July detected 89% plants infected with the pathogens and the disease incidence at 70%.

A re-infection test revealed that healthy Ponkan trees express HLB symptoms 2.5 years after planting. Among 92 plants tested, 32% plants were infected, six months later, 55%, and a year later 80% with a 33% disease incidence.

B. The Citrus Brown Aphid (*Toxoptera citricidus* (Kirkaldy))

Citrus tristeza could be transmitted by *Toxoptera citricidus*. Information on this vector is meager. It is distributed in the southern provinces of mainland China, Taiwan, Korea, Japan, India, Sri Lanka, Philippines, Malaysia, Indonesia, Hawaii, South America, and Africa.

It occurs year round. However, they are most abundant in spring and autumn. When the citrus flushes the alate adults fly into the orchard, infesting the new shoots and producing nymphs by means of viviparity. The nymphs take 6-42 days to complete four molts before becoming adults. A female could produce 5-68 nymphs. The adults live for 5-25 days. Alate forms appear as a result of crowding. The host plants include Rutaceae and Rosaceae plants. The aphids mainly infest heart leaves and young foliage on a branch. They excrete honey dew which induces sooty mold growth.

There are many predators (Coccinellidae and Syrphidae) and one parasitoid wasp attacking this aphid. However, the natural enemies exert insignificant impact on its population.

Control of the Vectors

Both vectors first appear on the host plants during the spring flushing. Plants protected with methomyl or malathion sprays at 10-day intervals during March to May were not infected by HLB. In order to control both vectors at the same time, 44% Dimethoate EC, 50% Malathion EC, and 40.64% Cabofuran FP are recommended during citrus flushing period. It is reported that 10% monocrotophos brushed onto the bark at the base of a branch at the onset of spring flush could effectively control citrus leaf miner, aphids, and psyllids without harming their natural enemies. Meanwhile, inoculative release of the ectoparasitoid, *Tamatixia radiata* (Waterson), a nymphal parasitoid of the citrus psylla, on Murraya plants could successfully keep the psylla reservoir population at a low level.

Conclusion

Technological know-how and information holds the key to solving disease problem. However, when it comes to technology transfer and adoption by the users and keeping the integrated control program moving, other resources should be taken into consideration. First of all, we need enough qualified and organized manpower to do the job, especially in the production of pathogen-free healthy seedlings, the monitoring of vectors and early detection and destruction of the infected plants, the release of natural enemies, and the training of personnel involved, etc. In addition, we need policy support so that adequate funding and pertinent regulations such as the Seed and Seedling Act and the Plant Protection and Quarantine Act are enforced to facilitate implementation of the program. Laws are indispensable for the regulatory control of the pests, especially for the enforcement in the destruction of inoculum sources (the diseased plants and vectors) as well as for the certificate system of the pathogen-free healthy propagules. Finally, not only the control technology and information needs to be integrated and managed, manpower, funding, and regulations also need to be integrated and managed so as to expedite the solution of the problem.

References

Aubert, B.; Quilici, S. Biological control of the African and Asian citrus psyllids (Homoptera: Psylloiddea), through Eulophid and Encyrtid parasites (Hymenoptera:Chalcidoidea) in Reunion Island. In: Proc. 9th Conf. Int'l Org. Citrus Virol.: Univ. Calif. Press, 1984. pp.100-108.

Chien, C.C.; Chiu, S.C.; Ku, S.C. Biological control of *Diaphorina citri* in Taiwan. Fruits 44(7-8): 401-407, 1989. (Chinese)

Chien, C.C. Bionomics of *Tamarixia radiata* (Waterson): an ectoparasitoid of *Diaphorina citri* Kuwayana in Taiwan. PhD dissertation. National Taiwan Univ., 1992. 138p.

Garnier, M.; Daniel, N; Bove, J.M. The greening organism is a gram-negative bacterium. In: Proc. 9th Conf. Intern. Organization Citrus Virol: Univ. Calif. Riverside, 1998. pp. 115-124.

Huang, C.H.; Tsai, M.Y.; Wang, C.L. Transmission of citrus likubin by a psyllid, *Diaphorina citri*: J. Agr. Res. China 33(1): 15-72, 1984. (Chinese)

Huang, C.H.; Liaw, C.F.; Chang, L.; Lan, T. Incidence and spread of citrus likubin in relation to the population fluctuation of *Diaphorina citri*: Plant Protection Bull. 32:167-176, 1990. (Chinese)

Huang, C.H. Liaw, C.F. A proposed strategy for control of citrus likubin from ecological viewpoint in Taiwan. In: Proc. Symp. Res. Develop. Citrus Taiwan: Taiwan Agr. Res. Inst., 1995. pp. 177-185. (Sp. Publ. No. 51)

Kotzé, J.M. Control of citrus greening: an epidemiological approach. Citrus J. (July): 10-11, 1985.

Lin, S.J.; Ke, Y.F.; Tao, C.C. Bionomics observation and integrated control of citrus psylla, *Diaphorina citri* Kuwayama. J. Hort. Soc. China, 19(4): 234-242, 1973. (Chinese)

Su, H.J.; Chen, C.N. Implementation of IPM of citrus virus and greening (likubin) diseases. Food and Fertilizer Technology Center Suppl. No.1: pp. 3-11, 1991.

Tang, L.C.; Su, T.H. Rearing method and developmental stages of the citrus psylla, *Diaphorina citri* Kuwayama. Bull. Soc. Entomol. (National Chungsing Univ., Taichung), 17:27-33, 1984. (Chinese)

Tao, C.C. Insect Pests of Fruits and Vegetables: Chinese Culture Univ. Press, Taipei, Taiwan, 1980. (Chinese)

Tsai, Y.P.; Hwang, M.T.; Wang, H.C. *Diaphorina citri* on Murraya paniculata: Plant Protection Bull. (Taiwan, ROC) 26:285-287, 1984. (Chinese)

Wang, C.L. Ecological studies of Asiatic citrus psyllid (*Diaphorina citri* K.) with special reference to its spatial distribution. J. Agr. Res. China 30(4): 412-419, 1981.

Wang, L.Y.; Hung, S.C.; Lin, C.J.; Hung, T.H.; Su, H.J. Population fluctuation of *Diaphorina citri* Kuwayama and its transmission in likubin-infected citrus orchards in Chia-yi area. In: Proc. Symp. Research and Development of Citrus in Taiwan: 1995. pp. 187-208. (Chinese)

Wang, L.Y.; Huang, S.C.; Hung, T.H.; Su, H.J. Population fluctuation of *Diaphorina citri* Kuwayama and incidence of citrus likubin in citrus orchards in Chiayi area. Plant Prot. Bull. (Taiwan, ROC) 38: 355-365, 1996. (Chinese)

Xu, C.; Xia, Y; Li, K.; Ke, C. Study on latent period of pathogen of citrus Huanglungbin in citrus psylla, *Diaphorina citri* Kuw. Acta Phytopathologica Sinica 20 (1): 25-31, 1990. (Chinese)

Xu, C; Xia, Y; Ke, C. Study on the biology and control of citrus psylla. Acta Phytophylacica Sinica 21(1): 53-56, 1994. (Chinese)

Citrus Greening Control Project in Okinawa, Japan

Shinji Kawano
Okinawa Prefecture Agricultural Experimental Station Miyako Branch
Japan

Introduction

Since citrus greening disease causes severe damage to citrus production, Japan has taken precautionary measures to control the occurrence of the disease in the country. The Plant Protection Law has listed citrus greening disease as one of the important diseases and pests of citrus. However, despite the strict measures, citrus greening disease has been found in Iriomote Island and Okinawa mainland in 1986 and 1994, respectively. The northern part of mainland Okinawa is a major commercial citrus-growing area. In 1997, the Japan Government, in collaboration with the Okinawa Prefecture, started a citrus greening disease control project.

The status of the citrus greening disease and its control project in Okinawa is discussed in this paper.

Okinawa Prefecture

Okinawa Prefecture is located southwest of Japan, and consists of a chain of islands forming the shape of a bow and situated between Kyushu and Taiwan. It consists of about 160 small and relatively large islands (about 50 inhabited islands) covering about 1000 km from east to west and 400 km from south to north (Fig. 1). Okinawa is the only region in Japan located in the subtropical zones. Okinawa Prefecture covers a total area of 2267 km^2 and consists of four major islands: Okinawa mainland (the largest island with an area of 1201 km^2), Iriomote Island, Ishigaki Island, and Miyako Island.

Citrus production in Okinawa

The total citrus production area in Japan is about 110,300 ha. Okinawa has about 605.2 ha (Table 1). The citrus varieties found in Japan are Satuma (*Citrus unshu*), Tankan (*Citrus tankan*), Kabuchi (*Citrus kabuchii*) and Shiikuwasya (*Citrus depressa*) which are all native varieties. Shiikuwasya, the most common citrus variety, is widely distributed in Okinawa prefecture, and can easily be found in backyard farms. Commercial citrus fields are located in central and northern parts of mainland Okinawa.

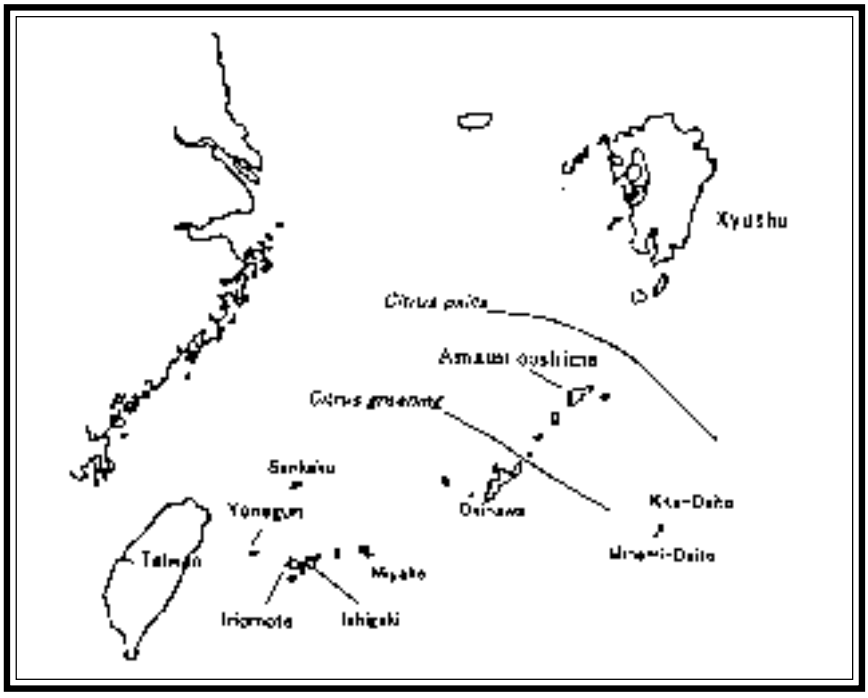


Figure 1. Location of Okinawa Prefecture, distribution of citrus greening disease and citrus psylla

Background

Insect vector, citrus pyslla (*Diaphoria citri*), is found in the southern part of Amami Islands in Japan. Though several citrus greening diseases surveys have been carried out in 1970, 1971, 1974, and 1980, results showed no incidence of infestation in Amami Islands and Okinawa Prefecture. Miyakawa and Tsuno, however reported the first citrus greening disease incidence on old Shiikuwasya trees at the Mitara and Oohara districts in Iriomate Island in 1988. The infected trees were immediately burned. At the same time, the government carried out surveys on Okinawa mainland, Iriomate Island, and Ishigaki Island but found no further incidence of infestation.

Five years later, a research team headed by Dr. Su (National Taiwan University) while surveying for the FFTC / ASPAC study "Natural enemies against vectors of virus and virus-like diseases of tropical fruits in Okinawa," found new infected trees at Sonai district (Dr. Su personal communication). In August 1994, Dr. Su and prefecture researchers found two more infected trees at the park on Itoman City in the southern part of Okinawa mainland.

Distribution of disease and vector insect

Initial detection methods utilized in Japan were grafting assay and hybridization assay using Taiwan University cDNA probe. Later, Polymerase Chain Reaction (PCR) assay using two primers became the standard detection method for citrus greening disease (Fig. 2).

Table 1. Citrus production in Okinawa Prefecture, 1995.

	C. Unshu		C. tankan		C. depressa		C. Kabuchi		Others		Total	
	Area (ha)	Yield (t)	Area (ha)	Yield (t)	Area (ha)	Yield (t)	Area (ha)	Yield (t)	Area (ha)	Yield (t)	Area (ha)	Yield (t)
Total	199.3	3084	258.1	2888	114.7	1815	23.2	216	10.3	97	605.8	8100
Okinawa:												
North	164.2	2680	244.3	2834	110.3	1802	22.5	213	9.7	92	551	7621
Middle	31.6	395	7.5	39	0	0	0	0	0	3	39.6	437
South	0.8	2	3.5	10	0	0	0	2	2	1	4.9	15
Miyako	-	-	-	-	-	-	-	-	-	-	-	-
Yaeyama	2.7	7	2.8	5	4.4	4.4	13	0.2	1	1	10.3	27

Data from Horticulture and Shipment in Okinawa Prefecture, 1997.
Others: *C. reticulata, C. gradis, C. oto, C. reticulata, etc.*

Survey results in 1996 showed that infected trees were more commonly found on backyards than on citrus orchards (Table 2 and 3). From 1995 to January 1998, citrus greening disease has been detected in 28 out of 53 cities, towns, and villages.

Surveys for distribution of vector insect, citrus psylla, were carried out again in 1995 and 1996 by the Plant Protection Office. Vectors were observed in Okinawa Prefecture and Amami Islands, Kagoshima Prefecture.

Table 2. Occurrences of citrus greening diseases on citrus orchard, 1996.

Island	Place	Variety	No. of Orchard	No. of Infected Trees
Okinawa	Nago City	*C. unshu*	2	4
	Nago City	*C. tankan*	1	2
	Onna Village	*C. unshu*	1	1
	Kin Town	*C. unshu*	2	9
Iheya	Iheya Village	*C. tankan*	1	1
Miyako	Gusukube Town	*Furtunella*	1	1
Iromote	Taketomi Town	*C. depressa*	1	1
	Total		9	19

Table 3. Occurences of citrus greening disease on house backyard, 1996.

Island	Place	Variety	No. of Point	No. of Infected Trees
Okinawa	Nago City	*C. depressa*	1	1
	Motobu Town	*C. depressa*	1	1
	Yonashiro Town	*C. depressa*	3	3
	Katsuren Town	*C. depressa*	2	2
	Ginowan City	*C. depressa*	1	2
	Urasoe City	*C. kabutili*	1	1
	Urasoe City	*C. depressa*	1	1
	Oosato Village	*C. oto*	1	1
	Itoman City	*C. depressa*	1	2
Izena	Izena Village	*C. depressa*	3	3
	Izena Village	*C. tankan*	1	1
Iheya	Iheya Village	*C. depressa*	2	2
Miyako	Shimoji Town	*C. depressa*	1	4
	Hirara City	*C. depressa*	1	2
Iriomote	Taketomi Village	*C. depressa*	10	11
	Taketomi Village	*C. unshu*	1	1
	Total		31	38

Prevention of Citrus Greening Disease Infection

Legislative control. So far, citrus greening disease has only been found in Okinawa Prefecture. Since August 1997, legislative control has been made to prevent the spread of the pathogen outside of Okinawa Prefecture.

Legislative control established were the following:
(1) Regulatory plants
Rutaceae *Poncirus trifoliata, Fortunella* sp. and genus *Citrus* living plants (exclusive of fruits and seeds)
(2) Regulatory pathogen and insect
Citrus greening bacteria and citrus psylla
(3) Regulatory area
South of latitude 27° 10'N, the southwestern islands (inclusive of Daito Islands, except to Yoron Island)

In transporting plants outside Okinawa Prefecture, regulatory plants are required to be inspected by a plant protection specialist. Jasmine orange (*Murraya paniculata*), a non-regulatory plant but a major host plant for vector insects is also subjected to inspection. It is necessary that no vectors are found on plants being transported.

Protection of Tankan Mother Stocks. Tankan fruits are one of Okinawa brands produced during winter (January to February) in Japan. The mother stocks and propagate stocks are managed by Okinawa Prefecture Agriculture Experiment Station-Nago Branch and Japan Agriculture Cooperative Association (JA), respectively. Most Tankan grafted nursery plants are propagated in Fukuoka and Kagoshima Prefectures located in Kyushu. Thus, since 1996, mother plants in Okinawa are regularly checked for citrus greening disease. These plants are maintained in a screenhouse isolated from vector insects. The nets prevent vector insect infestation because citrus psylla cannot pass through a 0.6 mm-mesh net.

Control of Vectors. Since 1996, a quarantine ban, prohibiting the transport of citrus trees and products from Okinawa has been set up. Transport of host plants of vector insects such as Jasmine orange, are also regulated to prevent the spread of the disease outside of Okinawa. In 1997, the application of pesticides, MEP (Sumithion) and DMTP has been recommended by Japanese government for vector control.

Control Project in Okinawa Prefecture

So far, citrus greening disease has only been found in Okinawa Prefecture. Okinawa Prefecture has started the control project for citrus greening disease with the support of the Japanese government. The objectives of project are: (1) to determine the distribution of the disease; (2) to prevent and control the spread of the disease; and (3) to eradicate the disease from the Okinawa Prefecture. Okinawa government has established an office responsible for the project and formulated on action program. Then, a control system was set up to implement the control project as illustrated in Figure 2.

The education team explains to people vital information about the disease and its control through meetings or leaflets, and collects information

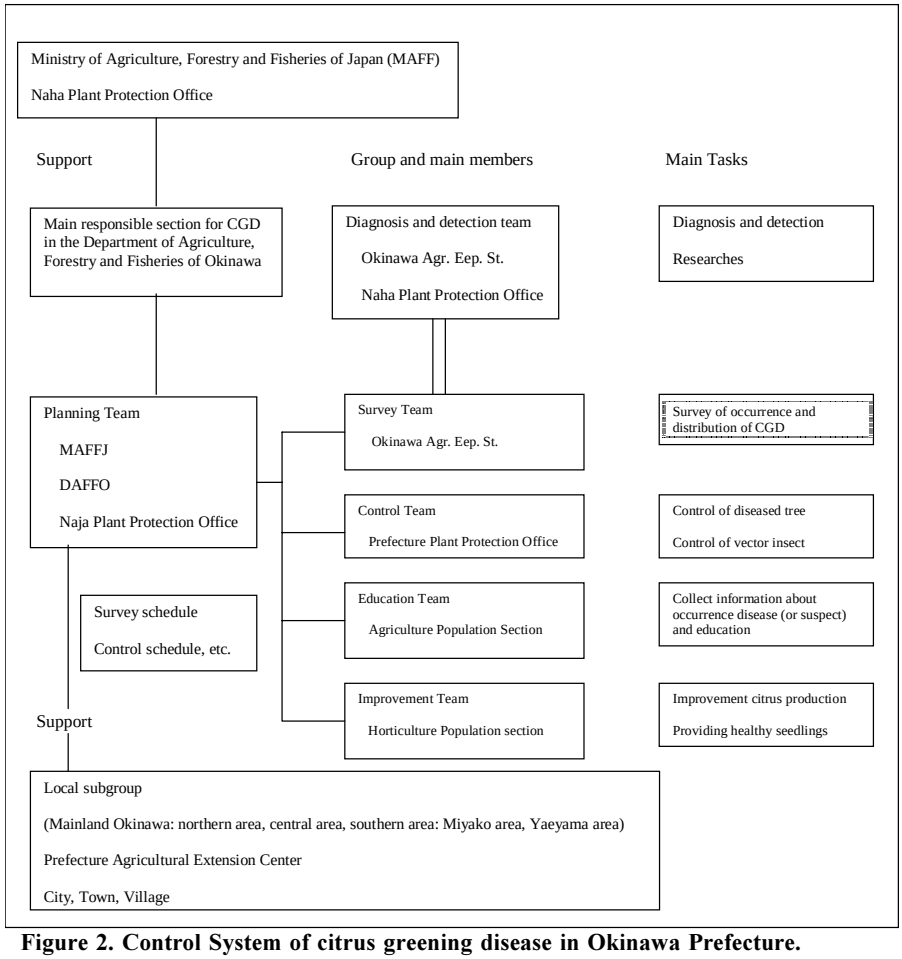


Figure 2. Control System of citrus greening disease in Okinawa Prefecture.

about infected citrus trees. Based on information gathered and survey results, the planning team discusses and decides on the conduct of large-scale survey and schedule the activity. The survey team then carries out the survey based on the plan. Collected samples are immediately assayed by the diagnosis and detection team. The control measures recommended are eradication of diseased trees and control of citrus psylla since the disease is transmitted through grafting or vectors and no chemical has been found effective. After diagnosing samples, control teams cut down infected trees after obtaining owner's consent. After cutting down the infected trees, herbicide is applied to stumps or burned up after digging trees. To ensure the area has been rid of the disease, survey teams carry out regular and careful survey on these points.

Problems on Control

The project's goal is to completely eradicate citrus greening disease from Okinawa, Japan. However, it is very difficult due to various factors. Among the problems faced in disease eradication is the difficulty in recognizing

the disease from symptoms alone. Similar symptoms may be caused by nutritional disorders, the presence of other diseases such as virus and others. Simple and high sensitive assay tools are needed for the project. Although PCR assay is a powerful tool for citrus greening disease, the handling is relatively difficult and the costs of instrument and chemicals are rather high. There is a need for more simple and inexpensive methods of assay or diagnosis for citrus greening disease.

Furthermore, the project needs more basic information about citrus greening disease under Okinawa conditions, such as infectious pressure, ecology of vector and disease transmissibility.

In controlling the disease, getting the consent of owners is sometimes difficult as some owners would still want to harvest from infested trees. Education at this stage is important.

Reference

Jagoueix, S.; Bove, J.M.; Garnier, M. The phloem-limited bacterium of greening disease of citrus is a member of the submission of the Proteobacteria. Int. J. Systs. Bacteriol. 44, 1994. pp. 379-386.

Kawano, S.; Su, H.J.; Uuhera, K. First report of citrus greening disease in Okinawa Island. Annals of the Phytopathological Society of Japan 63, 1997. pp. 256. (Japanese).

Miyakawa, T. Citrus diseases on Okinawa Islands. Syokubutu-boeki 26, 1972. pp. 241-244. (Japanese).

Miyakawa, T.; Tanaka, H.; Matsui, C. Studies on citrus greening disease in Southern Japan. In. Proceedings of the 6th Conference of the International Organization of Citrus Virologist, 1974. pp. 40-42.

Miyakawa, T., and Tsuno. Occurrence of citrus greening disease in the Southern Islands of Japan. Annals of the Phytopathological Society of Japan 55, 1989. pp. 667-670.

Miyatake, Y. Notes on Pysllidae form the Ryukyu Islands. *(Hemiptera: Homoptera).* Kontyu 33, 1965. pp. 171-189

National Fruit Tree Experiment Station. Special report on “Emergency research and study on simple diagnosis for citrus greening disease”. 1995. (Japanese).

Su, J.; Hung, T.H., Tsai M.C. Detection of the fastidious bacteria causing the Asian citrus greening by DNA probes. Abstract in Twelfth International Conference on Citrus Virology, New Delhi, India, 1993.

Tanaka, H.; Miyakawa, T.; Kagawa, M. Occurrence and distribution of stubborn-greening like citrus diseases and citrus pyslla on Kagoshime Prefecture. Syokubutu-boeki 24, 1970. pp. 514-518. (Japanese).

Toguchi, A.; Kawano, S. Distribution and control measures of citrus greening disease in Okinawa Prefecture, Syokubutu-boeki 51, 1997. pp. 565-570. (Japanese).

Session III

Management of Viral Streak in Banana and Plantain: Understanding a New Challenge

Ben Lockhart
University of Minnesota
USA

Banana streak virus (BSV), which causes viral disease of banana and plantain (*Musa* spp.), is the most widely distributed virus of these crops, and probably occurs wherever banana and plantain are grown. Although BSV was identified only in 1985, there is ample evidence which shows that it occurred in banana long before this date, and was often mistaken for cucumber mosaic virus (CMV), which causes a similar range of symptoms in banana, including foliar mosaic, cigar-leaf necrosis, and internal necrosis of the pseudostem. While the use of tissue culture is an important element in the management of the three major banana viral diseases, banana bunchy top (BBTV), banana streak, and cucumber mosaic, the viruses causing these diseases differ in certain fundamental respects, and an understanding of the characteristics of each of these viruses is essential to decision-making regarding investment in the productivity and deployment of virus-free planting material. In the context of disease management, the important considerations are distribution, epidemiology, and variability of the viral pathogen. These three characteristics have an important bearing on the final usefulness of virus-free planting stock.

With regard to distribution, BBTV differs from BSV and CMV in that it is absent from the New World, including the major banana-producing areas of Central and South America, and the Caribbean basin. Even in the Old World, BBTV has not been identified in major banana-producing areas of East and West Africa. In contrast, BSV and CMV occur in all of these areas. Hence, strict control of movement of BBTV-free planting stock is essential in order to prevent the movement of BBTV into virus-free banana-producing areas of the Americas and Africa.

The second aspect to be considered is epidemiology. Here, the contrast is between CMV on one hand and BBTV and BSV on the other. CMV infects a very wide range of cultivated plants and weeds and is transmitted by a variety of aphid vector species that also colonize or visit a wide range of plant species. As a result, spread of CMV into banana can occur from a diverse range of both wild and cultivated plants, and this spread can be mediated by a range of aphid species which feed on a variety of host plants. In contrast, BBTV and BSV occur almost exclusively in banana.

Likewise, the vector of BBTV, *Pentalonia nigronervosa,* occurs almost exclusively on banana, and although the mealybug vectors of BSV, for example, *Planococcus citri*, are highly polyphagous, the virus itself has a very restricted host range, and is known to infect only *Musa* and *Ensete.* Thus, the epidemiology of BBTV is dictated by the narrow host range of both virus and vector, while that of BSV is determined by the restricted host range of

both virus and vector. In contrast to CMV, BBTV and BSV move only from banana to banana, so that while re-infection of virus-free planting material by CMV can occur from a wide-range of sources and by a range of aphid vector species, re-infection of virus-free planting stock by BBTV and BSV can occur only from infected banana, and only over relatively short distances, since the vector of these two viruses do not disperse rapidly. As a result of these considerations, the use of virus-free tissue culture-derived planting stock as a disease management decision is more likely to be successful in the case of BBTV and BSV in comparison to that of CMV.

There is one aspect of the epidemiology of viral leaf streak of banana that merits mention and perhaps further investigation. This concerns the potential for sugarcane bacilliform virus (ScBV), which is closely related to BSV, to be transmitted from sugarcane to banana by mealybug vectors such as *Sacchariococcus sacchari* which are abundant on sugarcane and are capable of transmitting ScBV to banana. This results in symptoms similar to those caused by BSV infection. ScBV occurs widely in sugarcane germplasm collections and in sugarcane varieties in commercial production. There is no concrete evidence for the spread of ScBV from sugarcane to banana under natural conditions, but this possibility cannot be ignored.

The final characteristics to be considered are variability of the viral pathogen. Variability is important because, first, it determines the predictability of disease severity and crop loss when the pathogen moves into new areas of new crop varieties. Second, it determines the reliability of detection of the viral pathogen in planting material. In this respect, BSV differs sharply from BBTV and CMV. The latter two viruses exist as a limited number of biological variants characterized as producing severe or mild symptoms. All known isolates of BBTV and CMV can be detected reliably by serological (ELISA) or genome-based (PCR) methods. In contrast, isolates of BSV exhibit a very high degree of biological, serological, and genomic heterogeneity. A BSV isolate that produces mild symptoms in one banana cultivar may cause lethal systemic necrosis in another cultivar. Therefore, isolates of BSV cannot be neatly categorized as mild or severe, and introduction of BSV into new areas or new banana cultivars carries a high level of uncertainly and unpredictability with regard to symptom expression and potential crop damage. Equally important is the constraint that serological and genomic variability among BSV isolates place on reliability of viral detection and hence, on quality control in the production of virus-free planting material. When compounded with the fact that BSV symptom expression and virus titre fluctuate widely in individual plants and under different temperature conditions, the end result is that the indexing procedures for producing virus-free planting stock can be significantly less reliable for BSV as compared to BBTV and CMV.

There is, therefore, a risk of BSV in propagules derived from source plants which do not manifest apparent symptoms at a given period of the year, and/or in which BSV is not detected because of serological variability among BSV isolates. There is, however, a second problem of much greater potential importance. We have recently presented evidence that the process of tissue culture per se may result in *de novo* BSV infection in *Musa.* We had previously reported that segments of BSV DNA have become integrated into genomic DNA of a wide range of banana and plantain cultivars. Our data strongly support the hypothesis that under certain conditions these BSV sequences integrated into the *Musa* genome can give rise to BSV sequences.

This phenomenon has been documented from a wide range of *Musa* genotypes, including diploids (AA, AB), triploids (AAA, AAB ABB) and tetraploids (AAAA, AAAB). Interestingly, the majority of improved *Musa* cultivars, produced by the various breeding programs around the world, exhibit the tendency to produce BSV-infected propagules when virus-free mother plants are propagated *in vitro*. Fortunately, this tendency has not been observed in commercial dessert bananas which are selections from naturally-occurring cultigens, (e.g. Grand Naine, Williams). It should be recalled, however, that all dessert bananas is currently in commercial production. Our preliminary research on the frequency of BSV infection in tetraploid *Musa* hybrids bred for disease and pest resistance and improved yield, and in transgenic bananas carrying introduced genes for disease resistance, indicates that these processes (i.e. hybridization, and gene introgression) may contribute to the formation of integrated BSV sequences which are episomally-expressible during plant propagation by tissue culture. The observations suggest that genetic improvement of bananas, whether by conventional breeding or by genetic engineering, would be wise to recognize the possibility of *de novo* BSV infection arising from integrated viral sequence which may become active through recombination or rearrangement. Although there is so far only preliminary evidence for such an event occurring, the evidence is sufficiently convincing to suggest that any genetic manipulation of *Musa* should be approached with caution.

The information presented above serves to point out some biological, epidemiological, and molecular genetic characteristics of BSV that set it apart from BBTV and CMV and also present novel challenges in viral disease management through the use of tissue culture-derived virus-free planting stock. It also signals the potential danger of episomally expressible integrated BSV sequences arising during banana improvement programs. Current research at the University of Minnesota is focused on the characterization of BSV sequences integrated into the *Musa* genome, with a view to developing diagnostic methods which can reliably predict the possibility of integrated BSV sequences causing infection as a result of *in vitro* propagation, intraspecific hybridization, or transgenic introgression.

Reference

Comstock, J.C.; Lockhart, B.L. Widespread occurrence of sugarcane bacilliform virus in U.S. germplasm collections. Plant Dis. 74:50, 1990.

Dahal, G.; Hughes, J.d'A.; Thottappilly, G.; Lockhart, B.E. L. Effect of temperature on symptoms expression and reliability of banana streak badnavirus detection in naturally infected plantain and banana (*Musa* spp.) Plant Dis. 82: 16-21, 1998.

Diekmann, M.; Putter, C.A.J. FAO/IPGRI Technical guidelines for the safe movement of germplasm. *Musa* spp. (2nd ed.). FAO-IPGRI, Rome, Italy, 1996. 26 pp.

Jones, D.R.; Lockhart, B.E.L. Banana streak disease. *Musa* Fact Sheet No. 1, INIBAP, Montpellier, France, 1993.

LaFleur, D.; Lockhart, B.E.L.' Olszewski, N.E. Portions of the banana streak badnavirus genome are integrated in the genome of its host, *Musa.* Phytopathology 86: S100, 1996.

Lockhart, B.E.L. Purification and serology of a bacilliform virus associated with banana streak disease. Pathology 76:995-999, 1996.

Lockhart, B.E.L. Banana streak virus infection in *Musa*: Epidemiology, diagnosis, and control. ASPAC Food and Fertilizer Technology Center, Taiwan, Tech. Bull. 143: 11, 1996.

Lockhart, B.E.L.; Autrey, J.C. Occurrence in sugarcane of a bacilliform virus related serologically to banana streak virus. Plant Dis. 72:230-233, 1998.

Lockhart, B.E.L.; Olszewski, N.E. Serological and genomic heterogeneity of banana streak badnavirus: Implications for virus detection in *Musa* germplasm. In: Breeding Banana and Plantain for Resistance to Disease and Pests, Montpellier, France: CIRAD / IRFA / INIBAP, 1993.

Lockhart, B.E.L.; Ndowora, T.C.; Olszewski, N.E.; Dahal, G. Viral leaf streak of *Musa*: Investigating a novel virus-host interaction. In: Seminario Internacional sobre Produccion de Platano, Columbia: CORPOICA, 1998. 336 pp.

Ndowora, T.C.; Lockhart, B.E.L.; Olszewski, N.E. Relationship between integrated and episomal badnavirus genomic sequences in *Musa.* Phytopathology 87: S89, 1997.

Pathological and Molecular Characterization of Banana Bunchy Top Virus (BBTV) Strains in Asia

Hong-Ji Su
Li-Yu Tsao
Ting-Hsuan Hung
National Taiwan University
Taipei, Taiwan

Abstract

Molecular diagnostic probes such as monoclonal antibodies used in ELISA and primer pairs used in PCR analysis were developed for detecting and differentiating the strains of BBTV collected from Taiwan and the other Asian and Pacific countries. The symptom expression of the infected Cavendish banana plant was affected by the strains of BBTV. The isolates of BBTV strains isolated through aphid transmission were primarily characterized by symptom expression and grouped into four strains such as severe strain (S) causing distinct bunched atrophy leaves and dwarfing; intermediate strain (I) inducing moderate leaf atrophy and vein clearing; mild strain (M) showing symptomless or mild vein clearing; and latent strain (L) causing no symptom. The strains were further characterized by PCR amplification pattern with three primer pairs and grouped into six PCR-genotypes. The isolates of Taiwan severe strain were characterized into type I and II, and most of the S isolates belonged to type II. Most isolates of severe strain collected from the Asian and Pacific regions were characterized to be genotype II, however, the Malaysian isolate of S strain, belonging to type III, was quite distinct from the other severe isolates.

Introduction

The bunchy top disease (BBTV) has been the most common and destructive banana viral disease in Taiwan and some banana-growing areas in the Asian and Pacific regions. BBTV was first recorded in Fiji in 1891 and in Taiwan in 1892. Serious epidemics of BBTV occurred in Australia between 1913 and 1926. Legal control measures of the disease were established in Australia because of these serious incidence. Several occurrences of bunchy top outbreak during this century became the limiting factor for the banana industry in Taiwan (1900s, 1960s, and 1980s). This systemic disease is commonly spread through infected-banana suckers and tissue-cultured plantlets derived from infected mother stock, and transmitted by vector aphids (*Pentalonia nigronervosa*). Several countries such as Australia, Taiwan, and the Philippines have implemented extensive programs of inoculum elimination for checking the outbreaks. The disease has spread over Asia, South Pacific, and Africa. Amazingly, the disease has not reached Central and South America.

BBTV has been associated with the disease. The virus has 22 nm isometric virions with a coat protein of 21.1 kDa and a multicomponent of single-stranded DNAs (91.1 Kb) genome. Monoclonal antibodies (McAb) through hybridoma technique were prepared for diagnosing the disease and detecting BBTV in Taiwan. In the field disease survey by ELISA with McAb, several different strains of BBTV isolates showing different symptom expressions were found in Taiwan. The present study was made to differentiate the viral strains of Taiwan through symptom expressions and molecular characterization of ELISA assay with McAb and PCR analysis with different primer pairs. Some severe strain / isolates introduced from Asian and Pacific countries were also compared with Taiwan strains in molecular and symptom characterization.

Materials and Methods

Isolation and maintenance of Taiwan BBTV strain/isolates. Suckers of Cavendish banana plants showing bunchy top symptoms of different severity and found positive to ELISA test were collected from banana plantations over the island. Suckers of healthy-looking banana plants showing positive ELISA test were also collected and grown in pots under greenhouse condition for further use and observation of symptom expression. All the viral isolates were obtained through transmission by banana aphid (*Pentalonia nigronervosa*) to tissue-cultured (TC) plantlets of healthy Cavendish banana. A non-viruliferous aphid clone was used for separating virus isolates by aphid transmission. Fresh banana leaf tissues infected with BBTV were kept in moisten petri dish, and non-viruliferous aphids fed on the leaf piece for one-day acquisition feeding. Viruliferous aphids were allowed to feed on healthy TC plantlets for one-day inoculation feeding. The inoculated plantlets were sprayed with insecticide and kept in greenhouse for observation of symptom expression while some infected plants were selected for maintaining BBTV strain-isolates.

Banana leaf tissues were collected from banana plants showing typical BBTV symptoms grown in Asian and Pacific countries (Malaysia, Vietnam, Philippines, Guam, Fiji, Australia, and Hawaii) in recent years during FFTC survey trips and personal visit (Import permit No. 86-V-9). All foreign isolates of severe strains passed through aphid transmission were maintained in Cavendish plantlets kept in isolated greenhouse for further use in strain characterization. All foreign-isolate-infected plants were eliminated by herbicide spray after utilization in this experiment. The purified BBTV DNAs were kept in deep freezer for further use.

BBTV detection and strain characterization by ELISA and PCR (polymerase chain reaction). The ELISA (Enzyme-linked immunosorbent assay) with monoclonal antibody (2H6) is commonly used for detection of BBTV in banana and intermediate host plant tissue. The following three pairs of primers were used in PCR analysis for detecting BBTV and differentiating the viral strains. The three primer pairs (C1-CR, S-CR and SR-CR) were derived from common regions among sequences of DNA components of Taiwan severe strain such as stemloop (S-CR) right side of stemloop (SR-CR), left-side of stemloop (C1-CR).

BBTV primer pairs:

C1-CR:
F 5'-GGA AGA AGC CTC TCA TCT GCT TCA GAG AGC-3'
R 5'-CAG GCG CAC ACC TTG AGA AAC GAA AGG GAA-3'

S-CR:
F 5'-GGG GCT TAT TAT TAC CCC CAG C-3'
R 5'-AGC GCT TAC GTG GCG CAG CAC TAA CT-3'

SR-CR:
F 5'-TGT CGT CGG CGA CGA AGT CG-3'
R 5'-GGA CAT CCT CCT TCA GAA GAG AGA-3'

PCR cycles for C1-CR primer, using Taq polymerase, BRL: 94°C 4 minutes; 50°C, 1 minute; 72°C, 2 minutes for 1 cycle; 94°C, 1 minute; 50°C, 1 minute; 72°C, 2 minutes for 30 cycles. Last cycle, 72°C, 10 minutes, 4°C soaking.

PCR cycles for S-CR and SR-CR primer pairs were almost similar to the cycle for C1-CR primer except when annealing temperature was 60°C.

The PCR products were subjected to electrophoresis in 1.4% agarose gel running to 100v for about 30 minutes with the markers (Bromophenol blue).

Results and Discussion

Symptom expression of BBTV strains. The symptom expression of infected Cavendish banana plants were affected by strains of BBTV (Table 1). Severe strain (S) induced severe dwarf and typical bunchy top symptoms including slender atrophy leaves with marginal chlorosis, and vein clearing of different degrees, i.e. S-2 isolate of Taiwan S strain caused moderate vein clearing (VC), and S-3 isolate caused mild VC; while S-My-3 isolate of Malaysia severe strain induced distinct severe VC. The isolates of severe strain might be differentiated into at least three substrains according to the different severity of vein clearing and green streak in symptom expression. Intermediate strain (I) of Taiwan BBTV caused moderate stunting, mild leaf atrophy, bunchy top symptoms, and moderate vein clearing. The banana plants infected with mild strain (M) produced no distinct symptom which looked healthy, but very mild vein clearing was developed in some leaves (Fig. 1). Some healthy-looking plants were examined to be infected with a latent strain (L) of BBTV by showing mild positive reaction to ELISA test. The isolate of L strain were transmitted by banana aphids.

Molecular characterization of BBTV strains. In the preliminary test, PCR amplifications with different primer pairs was followed by electrophoresis analysis of PCR products. DNA components of the various BBTV strains as template bore different amplification patterns of PCR. It was assumed that the different strains causing different symptoms might be differentiated by PCR analysis with the selected primer pairs at molecular level in nucleotide sequences. Some representative isolate of the above mentioned BBTV strains were subjected to the PCR analysis with the selected three primer pairs (C1-CR, S-CR, and SR-CR). Each representative isolate of the strains developed

Table 1. Symptoms expression of different BBTV strains/isolates in Cavendish banana plants.

BBTV	Symptoms[b]				
Strain / isolates	Vein Clearing	Green Streak	Leaf atrophy	Bunchy Top	Stunting
S-2	++	++	+++	+++	+++
S-3	+	++	+++	+++	+++
S-My-3	+++	+++	+++	+++	+++
I-1	++	±	+	+	++
M-1	±	±	±	-	-
L	-	-	-	-	-

a.) BBTV strain isolates: S, severe strain; I, intermediate; M, mild strain; L, latent strain. My, Malaysian isolate of a severe strain.
b.) Symptom type index: -, no symptom; ±, very mild; +, mild; ++, moderate; +++, severe.

Figure 1. Symptom expression of Cavendish banana plants infected with BBTV strains.

A. Cavendish banana plants inoculated with such different BBTV strains as severe strain, S-2 isolate (A), intermediate strain, I-1 (B), mild strain, M-1 (C), and latent strain, L-1 (D).
B . Cavendish leaves infected with S-3, My-S3, My-S3, S-2, I-1, M-1, and L-1 isolates of BBTV strains (from right to left).

different patterns of PCR amplifications specific to template DNA of each strain (Table 2). The size of all PCR products of the strain DNAs with the three primer pair had similar length of about 1.1 kbp owing to the circular strands of virus genomic DNAs (1.1 kbs).

The different PCR patterns (Table 2) were designated to six PCR-genotypes (I to VI), since the annealing of each primer matching for the complementary sequence site of template DNA strand of each specific strain. The three representative isolates of severe strain (S), S-2, S-3, and My-S3 were differentiated into three different PCR genotypes, i.e. S-2 being amplified with the three primer pairs, was designated to type I (+/+/+); S-3 reacting with S1-CR and S-CR primers, belonged to Type II (+/+/-); and My-S3 reacting with S-CR primer only, designated to type III (-/+/-). The two

Table 2. Differentiation of BBTV strains by PCR with different primer pairs, showing six genotypes of virus strains in amplification patterns.

Primer Pairs	Strain Isolate of BBTV						
	S-2	S-3	My-S3	I-1	M-1	L	H-ck
	PGR-genotype						
	I	II	III	IV	V	VI	
C-1-CR	+++	+++	-	+++	+++	+	-[a]
S-CR	+++	+++	+++	-	++/+[b]	-	-
SR-CR	++	-	-	++	++	-	-
ELISA Index	++++	+++	-	++	+	±	-

a.) The different PCR amplification patterns shown by electrophoresis analysis of PCR products in 1.4% agarose gel: -, no amplification; Relative amount of positive amplification shown by density of electrophoretic bands of DNA products: + low, ++ moderate, and +++ high products.
b.) I Kb / 0.5 Kb.

isolates of Taiwan severe strain strongly reacted with the monoclonal antibody (2H6) in ELISA assay, while no ELISA reaction was demonstrated with Malaysian isolate of severe strain (My-S3). The isolates of Taiwan severe strain might be differentiated into two substrains as types I and II (Fig. 2A).

The isolates of intermediate (I) strain obtained through passage of aphid transmission derived PCR pattern of genotype IV (+/-/+) with DNA template strongly reacting with CI-CR and SR-C primer pairs, but was not amplified with S-CR primer pair. The DNA template of mild strain (M) was amplified in PCR with the three primer pairs by producing dense DNA bands of 1.1 kb in addition to a 0.5kb DNA thinner band with S-CR primer. The short DNA fragment was proven to be possibly defective interfering DNA fragment having sequence homology with a parent component (Fig. 2B).

Symptomless isolates (L) of BBTV were obtained from healthy-looking field banana plants which were examined to be infected with

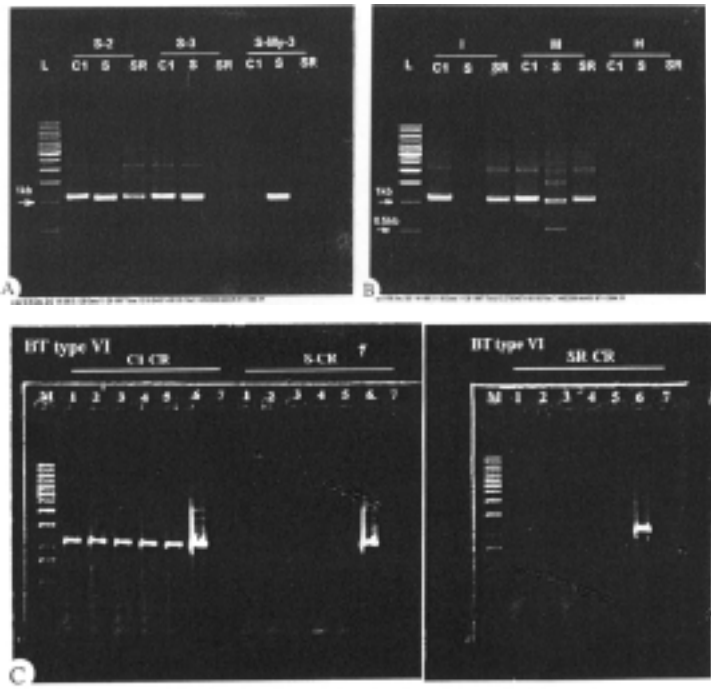


Figure 2. Molecular differentiation of BBTV strains by PCR with three primer pairs (C1-CR, S-CR, and SR-CR) followed by electrphoresis analysis. A. Electrophoresis analysis of PCR products from severe isolates (S-2, S-3, and S-My-3); B. Electrophoresis analysis of PCR products from intermediate strain / isolate (I), and mild strain / isolate (M). H, healthy CK; C. Electrophoresis analysis of PCR products from latent strain / isolates, 1. HC-10-4 isolate from *Hedychium coronarium* (1+C), replicated in HC; 2. HC-10-4 isolate in Cavendish.; 3. Alp-1-1 isolate from *Alpinia speciosa* (Alp).; 4 Alp-1-1 isolate, in Cavendish; 5. Latent isolate froma strain complex with S-3 severe isolate, in Cav.; 6. Severe isolate S-2, in Cav.; 7. H-CK, Cav.

BBTV by ELISA test. The L isolates were separated from BT-diseased banana plants co-infected with severe strain through aphid transmission.

These symptomless isolates were designated to be a latent (L) strain which derived genotype VI of PCR pattern (+/-/-). Their templates were amplified I-CR primer only (Fig. 2).

Molecular characterization of BBTV severe strain/isolates from foreign countries in Asian and Pacific region. Several isolates of BBTV severe strain were collected from bunchy top infected banana plants in the banana-growing countries like East Malaysia, Vietnam, Philippines, Australia, Fiji, Guam, and Hawaii. Most of the foreign severe isolates showed typical bunchy top symptoms with mild to moderate vein clearing in the original infected plants, and Cavendish TC-plants artificially infected by aphid transmission. Those isolates except Malaysian isolate strongly reacted with McAb (2H6) prepared against Taiwan severe strain of BBTV in ELISA assay. Most isolates characterized by PCR with the three primers, were found to be PCR genotype II. It was assumed that BBTV severe strain of Type II was commonly distributed not only in Taiwan but also in the Asian and Pacific-wide areas (Table 3). Only the Malaysian isolate of severe strain, My-S3 did not react with Taiwan McAb in ELISA but was amplified significantly with S-CR primer only by deriving PCR pattern of genotype III (-/+/-). This Malaysian severe isolate was the strain of BBTV quite distinct from the other Asian and Pacific S-strain isolates. Its sequence of DNA component was found quite different from the sequences of BBTV DNA components so far reported (unpublished data).

Table 3. PCR and ELISA reaction patterns of severe strain isolates from foreign countries in the Asian and Pacific regions.

Countries and BBTV isolates	Symptom	ELISA	TCR			PCR
			CI-CR	S-CR	SR-CR	Genotype
Malaysia						
S-My-3	BT/S	+	-	++	-	|||
S-My-3-PN-1	BT/S	-	-	++	-	|||
Vietnam						
S-VN-PN-1	BT/S	+	++	++	-	||
S-VN-PN-2	BT/S	+	++	++	-	||
Fiji						
S-FJ-PN-1	BT/S	|	+++	|||	-	||
S-FJ-PN-2	BT/S	|	+++	|||	-	||
Guam						
S-GM-PN-1	BT/S	|	+++	|||	-	||
Australia						
S-As-PN-1	BT/S	|	+++	+	-	||
S-As-PN-2	BT/S	|	+++	++	-	||
Philippines						
S-Fi-PN-1	BT/S	|	|||	+++	-	||
S-Fi-PN-2	BT/S	+	||	+++	-	||
Hawaii						
S-HW-1	BT/S	+	++	+++	-	||
S-HW-PN-1	BT/S	|	|||	|||	-	||

References

Dale, J.L. Banana bunchy top: an economically important tropical plant viral disease. Advances in virus research 33:301-325, 1987.

Diekman, M.; Putter, C.A.J. *Musa* spp. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. No. 15, 1996.

Hafner, G.J.; Harding, R.M.; Dale, J.L. A DNA primer asociated with banana bunchy top virus. J. Gen. Virol. 78:479-486, 1997.

Harding, R.M.; Burns, T.M.; Dale, J.L. Virus-like particles associated with banana bunchy top disease contain small single-stranded DNA. J. Gen. Virol. 72: 225-230, 1991.

Magee, C.J.P. Investigations on the bunchy top disease of bananas. Counc. Sci. Ind. Res. Bull. 30: 67, 1927.

Su, H.J.; Wu, R.Y; Tsao, L.Y. Ecology of banana bunchy top virus disease. In: Proceedings of the International Symposium on Recent Developments in Banana Cultivation Technology, Los Baños, Philippines: INIBAP-ASPNET, 1992. pp. 308-312.

Sun, S.K. Studies on the bunchy top disease of banana. Sec. Publ. Coll. Agric. 10: 82-109, 1961.

Tsao, L.Y.; Hung T.H.; Su, H.J. Differentiation of banana bunchy top virus (BBTV) strains by polymerase chain reaction (PCR) analysis with different primer pairs (abs). Plant Pathol. Bull. 6:217, 1997.

Wu, R.Y.; Su, H.J. Purification and characterization of banana bunchy top virus. J. Phytopathol. 128: 153-160, 1990.

Wu, R.Y.; Su, H.J. Production of monoclonal antibodies against banana bunchy top virus and their use in enzyme linked immunosorbent assay. J. Phytopathol. 128: 203-208, 1990.

Wu, R.Y.; You, L.R.; Soong, T.S. Nucleotide sequence of two circular single-stranded DNAs associated with banana bunchy top virus. Phytopathology 84: 952-958, 1994.

Yeh, H.H.; Su, H.J.; Chao, Y.C. Genome characterization and virus associated dsDNA components of banana bunchy top virus. Virology 198: 645-652, 1994.

The Impact of Tissue-cultured Plants in the Ongoing Eradication and Rehabilitation Program in the Philippines

Lydia V. Magnaye
Bureau of Plant Industry - Davao National Crop Research and Development Center (BPI-DNCRDC)
Davao City, Philippines

Abstract

Micropropagation by shoot culture technique has been developed for mass propagation of banana. This is now utilized as an integral component of the on-going eradication and rehabilitation program in the Philippines for mass production of healthy planting materials as a control approach to viral diseases, commonly spread through propagative materials.

With the widespread occurrence of viral diseases caused by banana bunchy top virus (BBTV) and Banana bract mosaic virus (BBrMV) obtaining disease-free field-grown suckers as planting materials is increasingly difficult. This concern underscores the need to incorporate viral indexing into the micropropagation system to ensure the virus-free status of the tissue culture derived plants.

Tissue cultured plants in virus disease control

Banana bunchy top disease remains to be a major threat to both local and export banana production in the Philippines. The disease is caused by a virus (banana virus) transmitted between plants by a vector, banana aphid, *Pentalonia nigronervosa*, an inhabitant to banana plant and other *Musa* species. Farm-to-farm spread of the disease is primarily through infected or diseased planting materials thus tissue culture now has become indispensable for mass production of virus-free planting materials.

The use of healthy planting materials and destruction of infected or diseased plants essential for the control of bunchy top is by no means a new approach. This has been practiced as early as 1946 and has been found highly successful (Eastwood, 1946). Essentially this technology has remained a satisfactory control approach although efficient implementation is still difficult for most farmers to sustain as may be affected by their own values and attitude. There is however, a need to place more emphasis on virus indexing to be incorporated into the micropropagation system to ensure the virus-free status of banana tissue-derived plants for commercial plantings since viruses can be transmitted through the process of tissue culture (Ramos and Zamora, 1990, Smith, *et al*, 1993). However, indexed tissue cultured derived plants is practical only if isolated from infected plants and vector.

Disease-free and pest-free aspect of tissue-cultured plants are the driving force behind the industry's acceptance of the technology. However, reinfection rate recorded in several farms suggest that severity of BBTV

infection was related to the management of the farm and the eradication efficiency by diseased plants as observed in three particular small farms at different management levels.

Rate of banana bunchy top reinfection on indexed tissue cultured plants under three levels of management:

Well Managed	Medium	Traditional
5 %	20%	75%

It becomes quite apparent that tissue-cultured plant that has been indexed for viral disease are only practical if they can be grown in isolation from nearby diseased plants and insect vector.

Taking into account the present knowledge and capacity of the smallholder farmers to tackle the difficult problem on *Musa* viruses, it is not only technical but also socioeconomic considerations that must be kept in mind. There is a need for farmers' understanding on how to keep tissue-cultured plants free of viruses after planting. Emphasis must be on a sustained effort to reduce disease incidence to minimum and protect the tissue culture plants as long as possible from infection. Recent findings, (Kenyon, *et al*, 1998) identified BBTV and BBrMV as causing the most damaging viral diseases in smallholder plantings. For BBTV, aphid transmission are generally over relatively short distance or the vector does not disperse actively, so secondary spread within plantings would be limited with timely and efficient rouging of the diseased plants. Relative to these findings, the identified main sources of inoculum would be:

1) Abandoned and poorly managed stands in smallholder plantings
2) Re-growth of suckers from incompletely destroyed infected mat in larger plantations and even in smallholder planting.
3) Unrecognized infection of suckers or tissue culture-derived plants used to plant new areas.

Conclusion

The ongoing rehabilitation program piloted in one barangay for each of the four major banana growing provinces in the Philippines recognizes the weakness experienced in the dissemination of the technology as evidenced by most farmers' non-recognition and lack of understanding about the disease. There is a need to increase awareness about the value of management in keeping the plantation from disease infection and stimulating adoption of improved crop protection practices. This is where the industry and government has to work in partnership towards the protection of the Philippine banana industry against the spread and proliferation particularly of banana bunchy top and other serious diseases of *Musa*.

There is an urgent need for increased technology promotion and adoption directed towards preventing the spread of serious *Musa* viruses like bunchy top to sustain our globally competitive banana industry. This concern covers the following component to address:

1) Early intervention to prevent pest and disease outbreak development
2) Use of clean / healthy planting materials like tissue culture-derived materials
3) Planting of pest and disease resistant varieties if available
4) Employment of well-trained inspectors
5) Prompt eradication of diseased plants
6) Compliance with quarantine regulations on movement of planting material.

References

Damasco, O. P.; R.C. Barba. *In Vitro* Culture of Saba Banana *Musa balbisiana* cv Saba (BBB). Phil. Agric. 67 (3): 351-358. 1984.

Eastwood, H.W. 1946. Bunchy top disease of bananas controlled by cooperative efforts. The Agricultural Gazette. Nov. 1, 1946.

Dickman, M. and C.A.J. Putter. FAO / IPGRI Technical Guidelines for the Safe Movement of Germplasm. No. 15. Musa 2nd edition. Food and Agriculture Organization of the United Nations, Rome / International Plant Genetic Resources Institute, Rome. 1996.

Kenyon, L.; Warburton, H.; Magnaye L.; Herradura L. 1998. Epidemiology and control of banana virus diseases in the Philippines. In. Research Highlights under the Integrated Pest Management Strategy Area, 1989-95. (eds). Hillocks, R.J. and Eden-Green, S.J. Natural Resources Institute on behalf of Natural Resources International Ltd, Chatham, UK.

Magnaye, L.V.; R.R. Espino. Note: Banana bract mosaic, a new disease of banana. I. Symptomatology. The Philipp. Agric. 73: 55-59, 1990.

Epidemiology and Integrated Management of Abaca Bunchy Top in the Philippines

Avelino D. Raymundo
Narceo B. Bajet
Department of Plant Pathology
University of the Philippines Los Baños
Philippines

Abstract

Bunchy top, transmitted by the aphid vector *Pentalonia nigronervosa*, is the most destructive viral disease of abaca. Epidemics of the disease have devastated many plantations in the Bicol Region for more than half a century. The disease can be controlled by eradication but this approach has not yielded the desired results due to inadequate understanding of the dynamics of the abaca-virus-vector relationship. The current collaborative program between the Fiber Industry Development Authority (FiDA) and the University of the Philippines Los Baños (UPLB) aimed at managing bunchy top with a holistic approach, is discussed in this paper.

A. Importance of abaca

Abaca or Manila Hemp (*Musa textilis* Nee) of the family *Musaceae* is indigenous to the Philippines. At the time of Ferdinand Magellan's landing in Cebu in 1521, the weaving of abaca fiber was already widespread in the islands.

The Philippines supplies about 84% of the total world abaca requirement, Ecuador satisfies the remaining 16% (FiDA, 1991). Total Philippine abaca export earnings from 1986 to 1990 was US$ 255.5 M (FOB), a yearly average of US $ 51.1 M, and is steadily increasing due to growing world demand.

Abaca is planted mainly as a source of fiber, either raw, yarns and fabrics and fibercrafts, or cordage and pulp. It is used for the production of specialty papers like currency notes, meat casings, tea bags, and capacitor papers.

Meeting the increasing demand for abaca fiber has presently been difficult due to declining productivity. This decline is due largely to diseases, primarily bunchy top in the Bicol Region and mosaic in Easter Visayas. The average yield per hectare in Bicol has dropped to 300 kg while the national average is 750 kg.

B. The abaca bunchy top

Abaca bunchy top in the Philippines was first reported in 1915 in Silang, Cavite (Ocfemia, 1927). In this province, and adjoining provinces of Laguna and Batangas, abaca was widely planted before and after World War I, at which time, the disease became rampant. In 1928, 12,000 ha of abaca were wiped out in Cavite (Calinisan, 1938). In 1930, the disease was also observed in Sorsogon Province in the Bicol Region.

Bunchy top was reported existing in the Davao area in 1937 at a time when large plantations of abaca were under the control of the Japanese.

The disease appears to have been left unchecked in the Bicol Region since the end of the Second World War. As recent as in 1980, it was observed already widespread in the area. Many areas, before and after this date, have been totally destroyed leaving only remnants of plantations. Large plantations, undoubtedly containing the disease, were abandoned due to deteriorating peace and order situation brought about by the insurgency problem. The presence of these distinct disease hot spots, untouched by any cultural intervention, has hastened the spread of the disease. Many of these abandoned bunchy top-affected areas still exist today.

Bunchy top is undoubtedly the single most important biological constraint affecting abaca. Its importance can be deduced from the thousands of hectares abandoned due to its onslaught. It is another matter, however, to quantify losses in relation to incidence or severity. No specific accounts pertaining to losses are readily available. Not even a reliable disease distribution pattern with corresponding incidence data exists. Bits and pieces of information regarding the dynamics of bunchy top and losses, mostly qualitative accounts, are available from a myriad of sources including observations of field technicians. A composite picture, however, has yet to be put together.

The presence of chlorotic streaks and transparent veins are initially the most reliable and characteristic symptoms of the disease. Under field conditions, affected plants become severely stunted and rarely produced meter-long pseudostems. They produce a large number of undersize suckers with small and narrow stiff leaves and curled-up sometimes necrotic margins and in general show the characteristic bunchy top symptoms. The associated dark-green appearance of the infected leaves and the restricted growth of the youngest emerging leaf noticeable as early as 14-18 days after inoculation are the early diagnostic symptoms of the disease.

Control of bunchy top has always been carried out primarily through elimination of diseased plants. This, however, has not been successful in slowing down disease spread. Infected plants are cut down instead of being rouged out. More often than not, there is no follow up to eliminate the regrowths that, as expected, are also infected. Farmers, likewise, are reluctant to destroy mature plants showing slight symptoms as these obviously can still be harvested. A stable type of resistance to bunchy top has yet to be found.

C. Epidemiology of abaca bunchy top in the Bicol Region

From an infected plant that is also infested with the aphid vector, *P. nigronervosa*, as a focal source of inoculum, the disease spreads through the movement of the vector carrying the virus. As the vector is a reluctant traveller, preferring to live in colonies on sheltered parts of the abaca plant, spread due mainly to active dispersal of the aphid is very slow.

Aphids spread the virus as they move from infected plants to healthy plants nearby. Winged forms develop when crowding of colonies occurs. Significant spread may then happen. Wind as an agent of dispersal can carry aphids for longer distances. However, vector dispersal most likely can carry aphids for longer distances. Also, vector dispersal most likely

occurs over very short distances, to another leaf on the same plant and to plants nearby.

The aphid vector, after successfully acquiring the virus from an infectious abaca plant, alights unto a new healthy host and is able to transmit the virus after a few hours. A bunchy top incubation time, from 14-18 days, can be ascertained by the dark green appearance of infected leaves and restricted growth of the youngest emerging leaf. Thereafter, chlorotic and transparent veins would be seen followed eventually by stunting and leaf bunching. Death of infected plants can happen after two years, hence, the onset and duration of the infectious period of the host have yet to be determined.

Environmental factors necessarily play a defining role in the dynamics of bunchy top of abaca. Both disease incidence and the occurrence and behavior of the vector are affected. Intensification and spread of the disease are related to the random flight of the vector which is influenced by seasonal changes and air temperature. At 10-15°C, bodily activities of the aphid are retarded along with their inclination to feed, thereby reducing the number of successful inoculations.

Despite the limited migratory tendency of the aphid vector, bunchy top has spread fast to adjoining areas from known localized sources of inoculum. A big part of this phenomenon has been attributed to weather disturbances during the rainy season. The Bicol Region lies in a typhoon path. Gusty winds during a typhoon certainly serve as carriers of vectors to great distances.

Abaca needs a certain degree of shading for optimum growth. This shading, which is common to many pathosystems, can provide a microclimate conducive to the continuation of the disease process.

The presence of ready alternate hosts of the virus and of the vector exacerbates the already favorable conditions inside big plantations for bunchy top explosive development. In addition to *Musa* spp. natural host P. nigronervosa include other species like *Costus glabra, Zingiber zerumbet,* cania (*Hedychium coronarium*), gabi (*Colocasia esculenta*), and *Caladium spp. P. nigronervosa f. caladii* has been mentioned as particularly important since *Caladium* spp. when in close proximity with abaca and even banana are usually heavily infested with the aphid. This host plant undergoes defoliation at particular times of the year trigerring migration of the alate form of the vector.

The aphid vector, in particular, has shown a tremendous capacity for producing a sizeable number of offsprings. Developmental time of the aphid ranges from 6-21 days with 4-5 nymphal instars. The reproductive rate at 26-29.1°C and 63-75% RH is 1-4 nymphs/female per day. The longest generation time is 25 days while the shortest is 20 days. The highest number of generation on abaca is 17.

In the Bicol Region, man apparently plays a significant role as an agent of dispersal of the vector and indirectly of the virus. In the course of normal farm activities, notably cultural operations and harvesting, man disturbs the natural habitat of the vector triggerring more than normal movement of non-alate forms and flight of the alate forms. These movements appear to be localized as indicated by current aggregated disease distribution in specific towns.

Two activities of man are usually suspected to have contributed significantly to the temporal and spatial development of bunchy top. In

the first case, in many abaca areas in the Bicol Region, bunchy top is conspicuous along pathways in plantations indicating the role that man's normal activities and movements play. In the second case, farmers normally collect abaca leafsheaths and leaves for handicraft purposes. These plant parts, many of them carrying viruliferous aphid vectors, are usually brought to other areas. In extreme cases, these are transported beyond provincial boundaries. It is quite apparent that a case of autonomous recurrent dispersal of both the vector and the virus has been occurring over many years through the agency of man. Although such situation has not been described in technical journals, a conjecture along this line concerning the current widespread development of bunchy top in the Bicol Region is not without scientific merits.

D. An integrated and sustainable approach to management of bunchy top

A non-holistic approach is a prescription for failure in managing a disease like abaca bunchy top. The very nature of the crop, being perennial, guarantees an uninterrupted availability of host for the virus and its vector. The disease expression assures that the virus is detected only after it has already successfully infected another plant. The presence of a vector that is able to survive in other hosts further complicates the situation. Previous programs has concentrated on eradication of affected plants showing obvious symptoms in areas where they are readily seen such as those close to population centers. Mountainous and other areas of rugged terrain were usually excluded for obvious reasons. Eradication was carried out simply by cutting down diseased plants. A follow-up to eliminate infected regrowths most often was not done due to limited resources. The disease, therefore continued, to intensify even when virus-free planting materials were planted since the inoculum did not only persist but has in fact multiplied many times over. In such kind of scenario, the resulting epidemic is even worse as the area has been disturbed prompting dispersal of the vector and consequently of the virus.

It appears, therefore, that the only way to successfully manage bunchy top is to consider all aspects of the abaca virus-vector relationship including social and political factors that at this point seen to stifle new approaches either technical or otherwise.

A system approach towards bunchy top management is discussed here. It is based largely on an ongoing collaborative project of the UPLB and FiDA. This approach considers the following aspects:

1. Epidemiology / dynamics of bunchy top
2. Mapping / disease distribution
3. Ecology and population dynamics of *Pentalonia nigronervosa*
4. Eradication and vector control by chemical spray
5. Detection / diagnosis
6. Tissue culture and replanting using virus-free planting materials
7. Training of technicians and farmers
8. Community education / farmer cooperation

1. Epidemiology/dynamics of bunchy top

Apart from aspects of the dynamics of abaca bunchy top mentioned above, the epidemiology of the disease is primarily a distillation of scattered pieces of information most of which are qualitative. Actual rates of infection from known foci of inoculum are not known. Disease progress curves and infection gradients, therefore, are not available. These are obviously of immense importance in charting the advance and direction of disease spread. Current studies are being done to determine the progression of bunchy top from localized foci. This is complemented by a corollary study on the population dynamics of *P. nigronervosa*. Weather factors prevailing during the conduct of the studies, likewise, are being monitored. Appropriate correlation analysis will be done.

2. Mapping and disease distribution

Eradication of bunchy top is currently being done in the Bicol Region. As disease distribution tends to be aggregated, there emerged over the years distinct hot spots. In many of these hot spots, abaca plantations have been wiped out. There are, however, remnants around which the disease appears to be spreading at a very alarming rate. For instance, there are areas in Sorsogon Province with percent incidence of 20 as of October 1997. Recent surveys of the same area yielded 40% incidence (FiDA Region 5 unpublished reports, 1998).

The current eradication drive requires that concentrations of bunchy top disease be pinpointed first. The strategy is to destroy these concentrations of bunchy top infected plants in order to reduce immediately the source of inoculum. At the same time, slightly affected areas will likewise receive same priority. The idea is to protect these areas before they become beyond rescue.

The whole region of Bicol is targeted for mapping. FiDA field technicians in all provinces of the region are being involved in this activity after appropriate training on correct diagnosis and accurate sampling of disease distribution.

The methodology being utilized in mapping is as follows: the prevalence of the disease is ascertained in every barangay (hamlet) of every town of all provinces of the region by extensive surveys. As surveys have been undertaken in the past, current surveys are aimed primarily at confirming prevalence. The degree of incidence in affected areas is determined by disease sampling. At the barangay level, three 1-hectare areas are selected. In each one-hectare area, the X-pattern of sampling is employed with three 10 x 10 m areas of each farm being rated for incidence. Data from the three areas are averaged to obtain the incidence for each one-hectare area. The mean of the three 1-hectare areas will be recorded as that for the barangay. The disease incidence for a particular town will be computed from the mean of the data from all barangays. The provincial average incidence is obtained from data from all towns.

3. Ecology and population dynamics of *Pentalonia nigronervosa*

Although the developmental stages of ***P. nigronervosa*** have been studied to a certain degree, the ecology and population

dynamics of this vector, as they relate to the dynamics of the disease, are far from being understood. As mentioned above, progression curves showing relationship between seasonal abundance of the vector and levels of bunchy top incidence and how these are affected by weather factors have not been determined.

The following strategies are being implemented: collection and identification of aphids, monitoring and recording of key environmental parameters, analysis of relationship between aphid population density and environmental factors, and determination of disease spread relative to aphid movement.

4. Eradication and vector control by chemical spray

The destruction of infected plants is a primary objective of the eradication program. Along with this, is an attempt to prevent the dispersal of the virus by reducing, if not eliminating, the insect vector. Several approaches have been tried.

Cutting of an infected plant at the base by using a bolo does not eliminate the virus source as infected regrowths will appear in due time as aforementioned. To dig the corms out, as sometimes practiced, is too laborious and costly not to consider the built-in resistance of farmers to eradication. Piercing infected plants with a bamboo stick previously dipped in a herbicide solution is a fast way of covering a wide area and has been done with much success. However, regrowths still appear due to inconsistency of the operation. Currently, the procedure being followed involves cutting down of diseased plants at the base, boring a hole at the center of the corm, and dropping 5 cc of herbicide. Regrowths do appear at the periphery of mats that are in an advanced state of decomposition but eliminating these has become a lot easier. This practice has been quite successful.

The abovementioned eradication procedure is complemented by chemical spray that is applied beforehand and is intended to kill the vector before it is disturbed and has the opportunity to escape. This spray is directed towards the bunchy top affected area and its immediate surrounding.

The current approach involves first the targeting of identified “hot spots”, local concentrations of disease, in order to drastically reduce the source of inoculum in the region. Thereafter, eradication will be directed at adjoining areas. At the same time, areas that have not been affected will be protected. This protection takes the form of early monitoring and prompt removal of infected plants. Although bunchy top can be found all over the Bicol Region, there are areas that somehow have remained apparently disease-free. Whether abaca plants in these areas are bunchy top virus-free or not has not been determined.

5. Diagnosis by serological techniques

Bunchy top is suspected to be present in all growing areas of the Bicol Region. In the absence of distinct characteristic symptoms on the abaca plant, the risk of virus dispersal through transport of planting materials is high. The source of plants to be used in tissue culture has also been a problem lately as these sources have never

been confirmed to be virus-free. Moreover, tissue-cultured materials ready for planting in areas to be rehabilitated will have to be indexed for the presence of the virus before transport. Serological techniques are now being used to solve this problem. A diagnostic laboratory is being built to complement the already existing tissue culture laboratories. Several research scientists of FiDA have undergone training on serological techniques at the Department of Plant Pathology at the University of the Philippines Los Baños.

6. Tissue culture and replanting using virus-free planting materials

Tissue culture for mass production of virus-free planting materials has been utilized in previous FiDA programs on abaca rehabilitation. This technique is an integral component of the current replanting program. It is being strengthened by appropriate virus diagnostic steps as described above.

Two tissue culture laboratories of FiDA in the Bicol Region are tasked with producing virus-free materials for replanting in areas that have just been subjected to bunchy top eradication. These laboratories are in Legazpi City and Sorsogon. The output from these laboratories has been augmented by the Tissue Culture Laboratory of the Department of Horticulture at the University of the Philippines, Los Baños. Deployment of these materials, unlike in previous programs, is in consonance with epidemiological principles. The "cleanliness" of areas to be replanted must be assured as it would be useless to deploy these virus-free materials where the virus still persists. As earlier mentioned, symptoms of bunchy top can be seen within a month after healthy abaca seedlings are exposed to virus inoculum.

The persistence and spread of bunchy top on abaca and on other host plants, such as banana, *Zingiber zerumbet*, and *Caladium* spp. over the years have aroused the suspicion that the virus is widespread and potentially suriving in asymptomatic plants. This has prompted less reliance on the usage of apparently healthy plants in replanting programs in the absence of reliable diagnostic techniques in remote program in the region. Tests, however, are being done regarding the efficacy of heat treatment in eliminting the virus in meristem and shoot-tip cultures.

7. Training of abaca technicians

Retooling of abaca technicians, many of whom have had little experience related to plant diseases, is a must in the current bunchy top eradication program. Over the last two years, training workshops have been conducted on topics ranging from agronomy of the abaca plant to social research aimed at understanding farmers' behavior towards the current situation in eradication of bunchy top. The main concentration of the training programs, however, is on the disease and its interaction with its vector and the abaca plant. Recently, a training on serological techniques for bunchy top and mosaic viruses was held for researchers of FiDA.

Breeding for resistance has been an integral part of these training activities. The current program on eradication, however, does not include host resistance as a genotype exhibiting this characteristic

in a consistent manner has yet to be found. The difficulty appears to be on a qualitative evaluation approach which is inconsistent with the quantitative nature of the disease.

8. Community Education, Farmer Vigilance and Cooperation

Vigilance and farmer cooperation are necessary components of a sustainable approach to managing bunchy top of abaca. As earlier mentioned, farmers have a natural tendency to resist change especially that concerning something they can only comprehend with extreme difficulty as in a systemic disease. It does not make sense to them to destroy a plant that, although diseased, is still a source of fiber yield. More so when an infected plant is asymptomatic. Imparting the logic of eradication and replanting with virus-free materials is a slow process that demands from the technicians lots of patience. The farmer's expectation of payment for eradicating bunchy top in his farm is, sometimes, exasperating. Here lies the need for continuing education to convince farmers of the gravity of the problem and of the need to understand it so that it can be dealt with properly. Farmers' willingness to cooperate is the key to the whole thing and so far this has proven to be quite difficult.

Farmers' training and print and broadcast media, among others, are being utilized towards this end. The availabililty of abaca farm technicians ready to help is a must. Interactions with landowners and other concerned parties such as processors, abaca product users, and traders are being held to optimize support towards the eradication of the disease.

References

Calinisan, M.R.; Hernandez, C.C. Studies on the control of abaca bunchy top with reference to varietal resistance. Philippine Journal of Agriculture 7:393-408, 1939.

Corcolon, M.R. Elimination of abaca viruses by chemotherapy and meristem culture. MS Thesis. University of the Philippines Los Baños, 1993.

FiDA. Annual Report. Fiber Industry Development Authority, Quezon City, Philippines, 1991.

FiDA. Sustainable research and management of abaca diseases in Bicol and Eastern Visayas. Project Operational Plan, Fiber Industry Development Authority, Quezon City, Philippines, 1995.

FiDA. Quarterly Report, Region 5. Fiber Industry Development Authority, Legazpi City, Philippines, 1998.

Lomerio, E.O. 1984. Comparative development of *Pentalonia nigronervosa* Coq. on five host plants. M.S. Thesis. University of the Philippines Los Baños, 1984.

Magnaye, L.V. Review of bunchy top researches in the Philippines. In: Proc. of the First National Symposium / Workshop on the Bunchy Top Disease of Banana and Abaca: Davao City, Philippines, 1989.

Ocfemia, G.O. Bunchy top of abaca or Manila hemp. I. A study of the cause of the disease and its method of transmission. American Journal of Botany 17:1-8, 1930.

Ocfemia, G.O. The bunchy top of abaca and its control. Philippine Agriculturist 20:328-340, 1931.

Ocfemia, G.O. Bunchy top of abaca or Manila hemp. II. Further studies on the transmission of the disease and trial planting of abaca seedlings in bunchy top devastated fields. Philippine Agriculturist 22:567-581, 1934.

Ocfemia, G.O. The abaca disease situation in Davao. Philippines Agriculturist 26: 229-236, 1937.

Raymundo, A.D. The bunchy top disease of banana and abaca: A reassessment, In: Proc. First PPS-SMD National Symposium on Pest and Diseases of Banana in the Philippines, Davao City, Philippines: April 1993.

San Juan, M.O. The bunchy top disease of abaca and banana in the Philippines: History and economic importance. In: Proc. of the First National Symposium / Workshop on the Bunchy Top Disease of Banana and Abaca, Davao City, Philippines: 1989.

Rehabilitation of BBTV - affected Areas in the Philippines: Experiences and Problems

Rene Rafael C. Espino
University of the Philippines
Los Baños, Philippines

B. S. Ligat
Benguet State University
La Trinidad, Philippines

L. V. Magnaye
BPI-DNCRDC
Davao City, Philippines

T. M. Espino
University of the Philippines
Los Baños, Philippines

Introduction

Banana (*Musa* sp.) is the most important fruit species grown in the Philippines. In 1997, 325,800 ha were planted producing 3.3 Mt valued at P 12.7 B (BAS, 1998). Through the years, it is consistently the number one export commodity among the different fruit species grown in the country. From 1993-1997, the Philippines had exported an average of 1.2 Mt of banana and its products valued at US$ 244.1 M.

Bunchy top disease was first reported in the Philippines in 1915 on abaca (*Musa textilis*) in Silang, Cavite (Ocfemia, 1927). By 1980, it was widespread in the Bicol region where abaca is widely grown (San Juan, 1989). Although, it was possible that bunchy top had existed in most abaca and banana areas, infection of bunchy top on banana (Lakatan) was first reported at Ballesteros, Cagayan in 1960 (Castillio and Martinez, 1961). By 1967, it started to become a problem in large farms of Cavendish at Subasta, Calinan, Davao City and in Tagum, Davao del Norte (Magnaye, 1989). Thereafter, it spread rapidly in the neighboring areas. San Juan (1989) stated that the disease had reached epidemic proportion since it was widespread in both the small and large farms attacking the different cultivars planted by the farmers such as Lakatan, Latundan, Bungulan, Saba, Cardaba, Giant Cavendish, Valery, Umalag, Morado, Señorita etc. Figure 1 shows the spread of the banana bunchy top disease (BBTD) in the country according to its degree of severity (Magnaye, 1996).

Control measures of this disease consists of a) early disease recognition and prompt eradication of infected plants; b) control of its insect vector, *Pentalonia nigronervosa*; c) use of virus-free planting materials; and d) quarantine for areas that are free from the said disease.

Rehabilitation of BBTD-Affected Areas

A research project entitled "Adoption of Control Strategies for Banana Bunchy Top Disease and Rehabilitation of Affected Areas" was initiated in 1994. Its primary objective is to prevent the spread of the disease and rehabilitate the affected areas. It focused on a community-based approach wherein small banana farmers were involved.

Four sites were selected to conduct the community-based piloting of control strategies for BBTD and subsequent rehabilitation effort. Selection of the project sites was based on the following criteria:

a. It must be situated in a banana growing area;
b. It must be affected by BBTD to a greater extent;
c. Cooperation of the local government officials and farmers in the community;
d. Willingness of the majority of the banana farmers in the area to participate and accept their responsibilities in the project;
e. Banana had contributed considerable portion to the family income; and
f. Accessibility of the area.

The sites are: 1) Brgy. Bayabas, Sablan, Benguet (Northern Luzon area); 2) Brgy. Guinhawa, Tagaytay City (Southern Luzon area); 3) Brgy. Bago-Oshiro, Davao City; and 4) Sitio Quimasod, Marilog, Davao City (Southern Mindanao area). Brgy. Bago-Oshiro was included to protect the germplasm collection of banana from the different Southeast Asian countries planted at the Davao National Crop Research and Development Center, Bureau of Plant Industry. Table 1 shows the characteristics of these sites.

In each site, visitations and consultations with barangay officials and banana growers were undertaken to determine their response. A rapid rural appraisal (RRA) was then undertaken to provide baseline information on the farmer cooperators and their management practices used in banana production. Afterwards, a training on the early diagnosis of BBTD through symtomatology and proper eradication methods was done. Initial eradication of affected plants on the farmer-cooperators farm was undertaken and monitored through actual farm visits. In addition, a resolution had been asked from the barangay council to allow any member of the community to eradicate any BBTD-infected plants.

Distribution of virus-free planting materials produced through tissue culture (shoot-tip culture) was done. Priority was given to farmer cooperators that immediately complied with the eradication of infected plants in their farm. A plant-now, pay-at-harvest scheme was followed. Moreover, they

Table 1. Characteristics of project sites in the rehabilitation of banana bunchy top disease-affected areas in the Philippines.

Criterion	Brgy. Bayabas, Sablan, Benguet	Brgy. Guinhawa, Tagaytay City	Brgy. Bago Oshiro, Davao City	Sitio Quimasod, Marilog, Davao City
Topography	Hilly-Mountainous	Flat-Slightly rolling	Flat	Rolling-Hilly
Cropping System	Mixed cropping with other fruits	Mixed cropping with coffee and ther fruits	Mixed cropping with other fruits	SALT-based
Cultivar	Lakatan	Inamibal Lakatan	Lakatan Cardaba	Latundan Lakatan
Area planted by farmers (ha)	<1.0-2.0	<1.0-4.0	<1.0-3.0	<1.0-2.0
Initial bunchy top infection	100	100	60-70	70-80
Contribution of banana to family income	High	High	High	Low to medium
Cultural management of banana	Low	Low	Low	Low
Accessibility	Accessible	Very accessible	Very accessible	Very accessible

were made to sign a promissory note to this effect. As part of the agreement, the farmer-cooperators will be responsible for the care of the plants. If the plants get re-infected with BBTD, these will be replaced. However, when the plants died due to their negligence or poor management practices, they were made to pay for the planting materials.

Regular visitations and farmer's meetings were held to monitor the performance of the plants in the field and discuss the progress and problems of the project. During farmer's meetings, technologies for the improvement of the farmer's cultural management practices were discussed for their adoption.

It was observed that frequent farm visitations and meetings made the farmer-cooperators more responsive and cooperative to the project activities.

Figure 1 shows the flow of activities for the setting-up and monitoring of the community-based piloting of control strategies for BBTD and rehabilitation of affected areas.

Accomplishment and experiences

During the first year of operation, few farmer-cooperators took part in the project due to: 1) farmer's reservation on the performance of the tissue-cultured planting materials; and 2) limited amount of available planting materials. It is important to note that when the farmers first saw the tissue-cultured plants (20-30 cm in height), majority were hesitant and expressed reservation. They ordered 5-10 plants / farmer for planting. Three to four months from planting, they saw that the plants were vigorously growing which led them to order more planting materials. Request of planting materials by the farmer-cooperators intensified when the plants started bearing large bunches. In addition, farmers from nearby barangays and other interested parties also requested planting materials and even volunteered to participate in the project.

1. Barangay Bayabas, Sablan, Benguet

Table 2 shows the summary of accomplishment of the project in the area. To date, there are 153 farmer-cooperators wherein majority are female. They were the ones who attended the farmer meetings / trainings and took care of their banana plantings. The major cultivar planted is Lakatan. With increased production of banana in the area, traders are now regularly coming to purchase their harvest. Fruits coming from the area command premium price in the market. Moreover, proliferation of fruit stalls (from 1 to 8) selling bananas along the national highway (Naguilian road) occurred. In the Baguio City market, two out of the eight retailers were now selling banana from this area.

Looking closely at the cultural management practices used by the farmer-cooperators in the area, it was found that those who adopted the recommended control strategies were able to produce banana profitably and had 10-14% re-infection rate. On the other hand, those farmer-cooperators which were lax in the eradication of re-infected plants had higher infection rate reaching 67-83% (Table 3). This shows that by continuous eradication of the infected plants and provision of virus-free planting materials, rehabilitation of the affected areas can be done and can be made productive again.

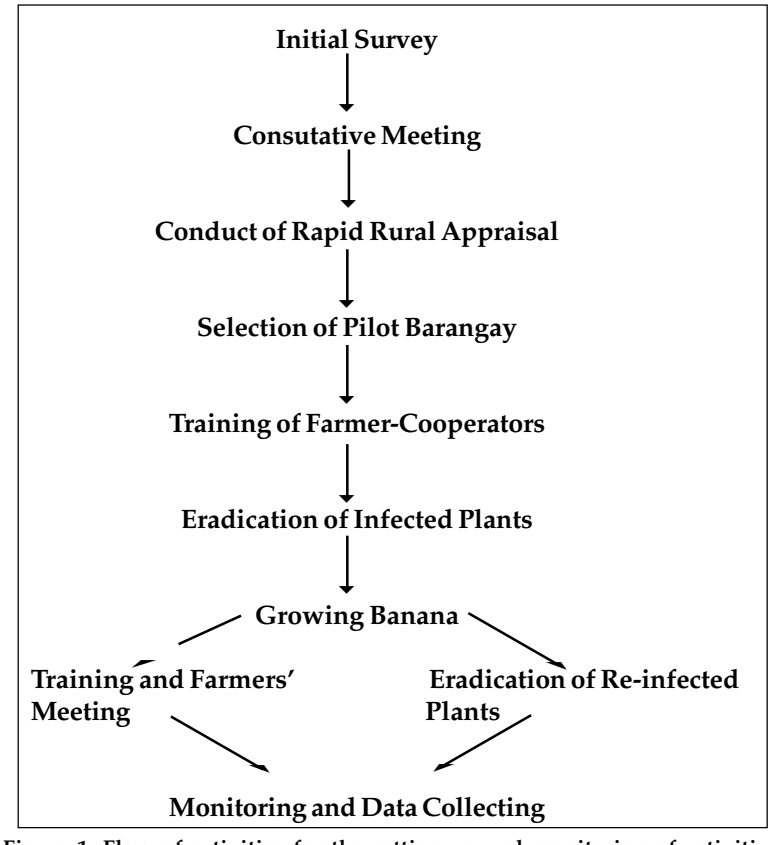


Figure 1. Flow of activities for the setting-up and monitoring of activities of the community-based piloting of control strategies for banana bunchy top disease and rehabilitation of the affected areas.

2. Barangay Guinhawa, Tagaytay City

Table 4 shows the summary of accomplishment of the project from 1994-96 in the area. A total of 54 farmer-cooperators participated in the project. The cultivars grown in the area are Inarnibal and Lakatan which were intercropped with coffee, coconut, and other fruit crops. The former cultivar is preferred in the area due to its short stature and early to harvest. Harvested fruits were sold in the fruit stall along the national highway. In most cases, the farmers- cooperators were selling their produce directly to the consumer due to their accessibility and nearness to the city proper.

The management practices done by the farmer-cooperators is shown in Table 5. Progressive farmers, which adopted the recommended practices for production and BBTD control, had the lowest re-infection rate(1-1.5%). This led to higher income to the farmer-cooperators concerned since more plants bore fruits and subsequently harvested.

Table 2. Result of the rehabilitation of banana bunchy top disease-affected area at Barangay Bayabas, Sablan, Benguet, Philippines.

Criterion	1994	1995	1996
Number of farmer-cooperators	23	84	41,433
Number of planting materials distributed*	3,000	26,658	18,600
Number of hectares planted*	4.0	24.3	72.3
Number of bunches harvested*	-	2,150	68,753
Value of the crops (US$)	-	7,225	153

* Includes those that were distributed to other interested farmers outside of the area.

3. Barangay Bago-Oshiro and Sitio Quimasod, Malagos, Davao City

The project summary of accomplishment in these areas is shown in Table 6. A total of 65 farmer-cooperators were involved in the project. In Brgy. Bago-Oshiro, the major cultivar planted was Cardaba which were planted along the borders of their farm and intercropped with other fruit trees. During the initial phase of the project, eradication of BBTD-infected plants was done by project personnel. Later on, this was done by the farmers themselves. Moreover, Lakatan are now planted in the area due to its high demand and price. At present, there is a great reduction in BBTD infection in the area from a high of 60-70% to 10-15%.

In Sitio Quimasod, Malagos, Latundan was planted together with other crops in a SALT-based cropping system. Later on, they shifted to Lakatan in monoculture due to the high demand by the traders / shippers in the area.

Table 7 shows the cultural management practices applied by the farmer-cooperators at Brgy. Bago-Oshiro, Davao City. Just like in the two sites, higher production was achieved by farmers which adopted the recommended technologies for production and BBTD control. BBTD re-infection rate is 1-2% for the progressive farmers while 37-40% for the traditional farmers. Farmers(intermediate) which followed to a certain extent the recommended practices obtained a re-infection rate of 10-12%.

In all of these four (4) sites, it was shown that areas that had been devastated by BBTD can be made productive to banana again. This can only be achieved with proper understanding of the disease by the farmer, provision of virus-free planting materials and the right attitude of the farmer.

Table 3. Cultural practices of farmer-cooperators in the rehabilitation of banana bunchy top disease-affected areas at Barangay Bayabas, Sablan, Benguet, Philippines.

Cultural Practice	Farmers		
	Progressive	Intermediate	Traditional
Land preparation	*ok*	*ok*	*ok*
Planting Distance			
3x3m	*ok*	*ok*	*ok*
3x5m	*ok*	*ok*	*ok*
3x8m	*ok*	-	-
Fertilization			
Basal	*ok*	*ok*	*ok*
Sidedressing			
1x a year	*ok*	*ok*	*ok*
2x a year	*ok*	-	-
Mulching	-	-	-
Weed control (manual)			
1x a year	-	-	-
2x a year	-	*ok*	-
3x a year	*ok*	*ok*	-
4x a year	*ok*	-	-
Deleafing			
1x a year	-	-	*ok*
2x a year	-	*ok*	-
3x a year	*ok*	-	-
Desuckering	*ok*	*ok*	*ok*
Propping	*ok*	*ok*	*ok*
Pest Control			
1x a year	-	*ok*	-
2x a year	*ok*	*ok*	-
3x a year	*ok*	*ok*	-
Harvesting			
Dehanding	*ok*	*ok*	*ok*
Transport	*ok*	*ok*	*ok*
Bunchy Top control	Early adaptor	Intermediate	Minimal
Percent re-infection	10-14	25-30	67-83
Number of plants/farmers	100-200	100-200	100-200
Income (US$)	725	249	37

4 - Farmers had done the cultural practice.
- - Farmers did not do the cultural practice.

Table 4. Result of the rehabilitation of banana bunchy top disease-affected area at Barangay Guinhawa, Tagaytay City, Philippines.

Criterion	1994	1995	1996
Number of farmer-cooperators	10	22	54
Number of planting materials distributed*	250	4,657	22,781
Number of hectares planted*	0.5	9.0	25.7
Number of bunches harvested*	-	180	3,200
Value of the crops (US$)	-	400	4,445

* Includes those that were distributed to other interested farmers outside of the area.

Problems encountered

There were many problems that were encountered during project implementation. The important ones are as follows:

1. Farmers' attitude. It is really hard to gauge the sincerity / cooperativeness of the farmers. One can always get a whole range of response. During meetings, everyone agreed on the different activities to be undertaken especially on the prompt eradication of BBTD-infected plants. However, upon field visitation, one can observed that this had not been done for one reason or another. Sometimes, these are the same persons who were vocal during the meetings and wanted to be given the priority in the distribution of planting materials. Personal approach through the "hiya" system to these farmers and peer pressure seem to work and make them do the things that were needed for a successful rehabilitation work. However, this requires a lot of time and effort on the part of the project personnel.
2. Lack of continuous support from the local government units(LGUs). An agricultural technician is assigned in the project sites as counterpart of the LGU. With the increasing demand for the expansion of the coverage of the project in other barangays, additional resources for the procurement of virus-free planting materials were needed as LGU counterpart. However, this had not been acted upon by the LGU officials. In some cases, the LGU's seem not to be concerned with the project activities.
3. Lack of virus-free planting materials. Tissue-culture derived planting materials were distributed to farmer-cooperators. The number of planting materials produced could not satisfy the demands since farmer-cooperators always increased their request at planting time. In addition, farmers from other localities also requested for planting materials. Farmer-cooperators also gave planting materials to their friends and relatives who were not within the scope of the project sites.

Table 5. Cultural practices by farmer-cooperators in the rehabilitation of banana bunchy top disease-affected areas at Barangay Guinhawa, Tagaytay City, Philippines.

Cultural Practice	Farmers		
	Progressive	Intermediate	Traditional
Land preparation	*ok*	*ok*	*ok*
Planting Distance			
3x3m	*ok*	*ok*	*ok*
3x5m	*ok*	*ok*	*ok*
Fertilization			
Basal	*ok*	*ok*	*ok*
Sidedressing 1x a year	*ok*	*ok*	-
2x a year	*ok*	-	-
3x a year	*ok*	-	-
Mulching	-	-	-
Weed control (manual)			
1x a year	-	-	*ok*
2x a year	*ok*	*ok*	-
4x a year	*ok*	-	-
Deleafing			
2x a year	-	*ok*	*ok*
3 x a year	-	-	-
4 x a year	*ok*	-	-
Desuckering			
2x a year	-	*ok*	-
4x a year	*ok*	-	-
Propping	-	-	-
Pest Control			
2x a year	-	*ok*	-
3x a year	*ok*	*ok*	-
4x a year	*ok*	*ok*	-
Harvesting			
Dehanding	*ok*	*ok*	*ok*
Transport	*ok*	*ok*	*ok*
Bunchy Top cotnrol	Early Adaptor	Intermediate	Minimal
Perecet re-infection	2-3	10-12	37-40
Number of plants/farmers	200	200	200-240
Income (US$)	791	319	23

4 - Farmers had done the cultural practice.
- - Farmers did not do the cultural practice.

Table 6. Result of the rehabilitation of banana bunchy top disease-affected area at Barangay Bago-Oshiro, and Sitio Quimasod, Marilog, Davao City, Philippines.

Criterion	1994	1995	1996
Number of farmer/cooperator	10	45	65
Number of planting materials distributed*	1, 500	16,348	32,934
Number of hectares planted*	3.5	13.1	44.6
Number of bunches harvested*	-	1,050	11,400
Value of the crops (US$)	-	767	20,267

* Includes those that were distributed to other interested farmers outside of the project site.

Conclusion

Proper disease management is the key to the control and rehabilitation of areas affected by BBTD. Large plantations are successful in controlling BBTD while maintaining their areas productive. They have the necessary resources to implement the proper control measures. Moreover, decision to do it or not is confined to few persons. For the small farms, it is often difficult since banana is grown in backyards and is only one of the crop grown by the farmer in his cropping system. Most often, he lacks the necessary resources to implement the necessary control measures.

Based on our accomplishment, rehabilitation of BBTD-affected areas can be successful in small farms provided the following conditions can be met: a) education of the farmers and agricultural technicians in the area on the disease, its symptomatology, insect vector, and control measures; 2) cooperation of the whole community in the prompt eradication of infected plants; 3) availability of virus-free planting materials; 4) availability of technologies to improve production; and 5) continuous support of concerned local government units.

Acknowledgment

The authors wish to acknowledge the financial support provided by the Department of Science and Technology (DOST) and Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD) in undertaking this research project entitled “Adoption of Control Strategies for Banana Bunchy Top Disease and Rehabilitation of the Affected Areas”

Table 7. Cultural Practices by farmer-cooperators in the rehabilitation of banana bunchy top disease-affected areas at Barangay Bago, Oshiro, Davao City, Philippines (case study).

Cultural Practice	Farmers		
	Progressive	Intermediate	Traditional
Land preparation	*ok*	*ok*	*ok*
Planting Distance			
3x3m	*ok*	*ok*	*ok*
3x5m	*ok*	*ok*	*ok*
Fertilization			
Basal	*ok*	*ok*	*ok*
Sidedressing			
1x a year	*ok*	*ok*	-
2x a year	*ok*	-	-
3x a year	*ok*	-	-
Mulching	-	-	-
Weed control (manual)			
1x a year	-	-	*ok*
2x a year	*ok*	*ok*	-
4x a year	*ok*	-	-
Deleafing			
2x a year	-	*ok*	*ok*
3x a year	-	-	-
4x a year	*ok*	-	-
Desuckering			
2x a year	-	*ok*	-
4x a year	*ok*	-	-
Propping	-	-	-
Pest Control			
2x a year	-	*ok*	-
3x a year	*ok*	*ok*	-
4x a year	*ok*	*ok*	-
Harvesting			
Dehanding	*ok*	*ok*	*ok*
Transport	*ok*	*ok*	*ok*
Bunchy Top control	Early Adaptor	Intermediate	Minimal
Percent re-infection	2-3	10-12	37-40
Number of plants / farmers	200	200	200-240
Income (US$)	791	319	23

4 - Farmers had done the cultural practice.
- - Farmers did not do the cultural practice.

References

Bureau of Agricultural Statistics. Selected agricultural statistics. 1998.

Castillo, B.S.; Martinez, A.L. Occurrence of bunchy top disease of banana in the Philippines. FAO Plant Prot. Bull. 9:74-75. 1961.

Ocfemia, G.O. Second progress report on bunchy-top of abaca or Manila hemp. Phytophathology 17:255-257. 1927.

Magnaye, L.V. Review of bunchy top researches in the Philippines. In 1st National Symposium / Workshop on Bunchy Top Disease of Banana and Abaca. 09-10 March, 1989, Davao City. Philippine Phytopathological Soc. 6-13, 1989.

Magnaye, L.V. Rescue and Conservation of the Southeast Asian Regional Banana Germplasm Collection. Annual Report. INIBAP / RECSEA, 1996.

San Juan, M.O. The bunchy top disease of abaca and banana in the Philippines: History and economic importance. In Proc. 1st National Symposium / Workshop on Bunchy Top Disease of Banana and Abaca. 09-10 March, l989. Davao City, Philippine Phytopathological Soc. 1-5, 1989.

Session IV

Status of Disease Management of Citrus in the Philippines: Focus in the Cordillera

Ceferino A. Baniqued
Bureau of Plant Industry -
Baguio National Crop Research and Development Center
Baguio City, Philippines

Introduction

The citrus industry in the Philippines once flourished in the 50s and 60s. Citrus used to be the fourth among the national priority crops. However, due to a major decline which occurred in the late 70s, particularly in the provinces of Batangas and Laguna, citrus was pushed out of the priority list. The decline of the citrus industry was attributed primarily to Huanglongbing, also called leaf mottling or greening disease. Prior to this, citrus fruits had a high demand in the domestic market and area expansion was notably fast. Massive propagation was done to cope with the demand for planting materials. However, in the process, the sanitation component was overlooked. Consequently, graft-transmissible diseases, particularly Huanglongbing and Citrus Tristeza, spread to other citrus-growing areas in the country. It is an established fact that the spread of disease in other citrus growing areas in the country was due to the transport of infected plant materials from affected areas particularly Batangas, which was the center of citrus nursery production in the past. Hence, nurseries play an important role in disease prevention and spread.

In the Cordillera region, citrus is increasingly becoming an important fruit commodity. Citrus production got a boost with the implementation of the Phil-German Fruit Tree Project from 1986-1997. Both citrus orchards and nurseries were established. Although in the Cordillera, commercial citrus nursery operation is still in its infancy. To date, there are about 23 nurseries. These were established under certain operating guidelines to ensure production of high quality planting materials.

There have been several attempts to rehabilitate the industry at the national level, however, its full implementation was hindered due to limited resources. Recently, the need for rehabilitation was revived and a Citrus Integrated Research and Development Program (IRDP) was packaged by PCARRD.

Common diseases of citrus in the Philippines

At present, there are eight virus and virus-like diseases of citrus reported in the Philippine. These are: Citrus Tristeza Virus (CTV), Huanglongbing (greening / leaf mottling), exocortis, psorosis, cachexia / xyloposis, tatterleaf, woody gall / vein enation, and bud union crease of sweet orange on rough lemon rootstock. CTV is very destructive to all sweet orange varieties such as Gayunan (the local mandarin grown in upper Kalinga and is popularly called

Kalinga or Balbalasang orange) and Pummelo. The severe strains of the virus induce stem pitting leading to stunting and production of small fruits (Ochasan *et al*., 1996 and Herradura *et al*., 1996).

Huanglongbing (greening) is the most devastating disease of citrus causing ultimate decline of trees. It is practically present in all citrus-growing areas in the country causing damage to almost all citrus varieties grown. The widespread occurrence can be attributed to the use of contaminated planting materials, which also carried insect vector (*Diaphorina citri* Kuway).

Exocortis viroid causes dwarfing and bark scaling on susceptible rootstocks such as the trifoliate and its hybrids. It was first reported on Zinkom causing bark splitting. The disease is a serious threat to the citrus industry in the highlands due to the predominant use of trifoliate hybrid rootstocks such as Troyer, Carrizo and Citrumelo.

Tatterleaf was reported to infect Philippines citrus by Su and Tsai (1990). It was detected on Calamandarin, pummelo and calamondin. The tatterleaf virus causes bud union crease of citrus budded on trifoliate orange and its hybrids (Miyakawa, 1980). Infected trees become stunted and easily break at the bud-union when there is strong winds. Similary, this virus is a serious threat to the highlands due to the predominant use of trifoliate and its hybrids as rootstocks.

Other than the graft-transmissible diseases, various diseases caused by fungi, bacteria and nematodes also plaque citrus production in the Philippines. The important diseases caused by fungal pathogens are powdery mildew, citrus scab, pink disease, melanose, anthracnose, stem-end rot, blue / green molds, and the diseases caused by *Phytopthora* spp such us root rot, foot rot, gummosis, brown rot, and shoot blight. Powdery mildew is a perennial problem in certain areas. In severe infections, massive dieback of shoot, defoliation, and fruit drop occur. Scab is also an important fruit blemishing disease, common on calamansi and lemons. Pink disease is also important as it girdles large branches leading to die back. Perhaps the most destructive among the fungal diseases is *Phytophtora*. It occurs from the nursery causing root rot on newly germinated seedlings leading to damping off and shoot blight on newly budded plants. In the field, it causes root rot, foot rot, and gummosis. Moreover, it affects fruits at near maturity causing brown rot leading to fruit drop and fruit rot later in storage.

The citrus bacterial canker is an important rind blemishing disease. In the field, severe infection causes defoliation and fruit drop. Infections on fruits render it unmarketable. Likewise, in the nurseries, infection causes severe defoliation.

Citrus is also affected by nematodes particularly *Tylenchulus semipenetrans* Cobb. Although nematodes do not kill trees, they contribute to the debilitation of trees, thus, slowing their productivity and eventually rendering them unproductive. According to Davide (1992), practically all citrus growing areas in the country are infested by plant parasitic nematodes. Nematodes are often unnoticed because the aboveground symptoms are not very specific. Their symptoms are more or less similar to those caused by other root attacking organisms and sometimes they are in asssociation with other diseases such that they are often overlooked leading to their enormous build up.

Detection methods for graft-transmissible disease

At present, both the facilities and expertise on detection of graft-trasmissible diseases of citrus are located at the Bureau of Plant Industry, particularly in Baguio and Davao National Crop Reseach and Development Centers. These centers also maintain clean citrus foundation materials that are regularly indexed against systemic diseases.

The different techniques being employed for the detection of graft-transmissble diseases of citrus include biological indexing, enzyme linked immuno sorbent assay (ELISA), and Polymerase Chain Reaction (PCR).

Biological indexing is used to detect the different virus and virus-like diseases. This involves the use of sensitive indicator plants such as Mexican or key lime for the detection of citrus tristeza virus, ponkan or szinkom seedlings for Huanglongbing / greening, and Etrog citron for exocortis. The procedure involves the graft-inoculation of the indicator plant with buds taken from suspected plant. Symptoms are later observed on the indicator plant. A modified-tissue graft called "side grafting" was developed by Magnaye (personal communication). This allows the continuous indexing of mother trees.

ELISA is also used for the detection of CTV using polyclonal or monoclonal antibodies. It is an important tool for a virus detection especially when testing for a large number of materials.

Recently, PCR was developed for the detection of citrus Huanglongbing / greening disease through DNA cloning and sequencing of the greening bacterium (Su and Huang, unpublished; Jagouiex *et al*, 1996; and Tian *et al*., 1996). At present, the Baguio National Crop Research and Development Center has the necessary equipment and trained staff to do this test. However, with the meager resources, the sustainability of the facilities is now in question. There is a need to sustain these facilities primarily for the indexing of foundation materials and provide service to commercial citrus nurseries. This is in line with the mandate of BPI to do plant material certification and ensure the production of high quality planting materials.

Disease management strategies

Integrated control of graft-transmissible diseases

1. Use of clean planting materials

The use of clean planting materials is the key component in the management of citrus diseases. This does not only mean freedom from systemic diseases but it also includes other important diseases like bacterial canker and soil-borne pathogens like *Phytopthora* and nematodes.

A planting material production system for citrus, initiated by the Phil-German Fruit Tree Project, is being implemented in the Cordillera region (Fig. 1). The basic step in the production of clean planting materials is the establishment of clean foundation stocks as the primary budwood source. At present, a modest collection of clean citrus foundation stocks is available at the Baguio National Crop Research and Development Center, Baguio City (Table 1). Majority of these were obtained from accredited Foundation blocks abroad, except the local cultivar, Gayunan, which was derived through shoot tip grafting (STG). STG is also being done for the other local citrus cultivars

with commercial importance. Clean stocks, particularly pummelo and mandarin, are available at the Davao National Crop Research and Development Center. These foundation plants are established under screenhouses to avoid contamination by insect vectors. Moreover, the trees are regularly indexed for systemic diseases and horticulturally evaluated to monitor genetic stability.

Table 1. Citrus foundation stocks at BNCRDC, Baguio City.

Common namea/Species	Cultivars
1. Sweet orange (*Citrus sinensis* (L.) Osb.)	Hamlin Trovita Salustiana Newhall Washington navel Atwood navel Gillette navel Olivia Valencia Navel late Navelina Ambersweet
2. mandarin (*Citrus reticulata* Blanco)	Ponkan Fortune Gayunan Hickson Glen Retreat SRA 600
3. *Citrus deliciosa* Ten	Commune Fina
4. *Citrus limon* (L.) Burm.	Fino Verna
5. *Citrus meyeri* Y. Tan	Meyer
6. *Citrus tankan* Hay	Tankan
7. *Citrus unshiu* Mak. Marc	Okitsu satsuma
8. *Citrus maxima* (Burm.) Merr.	Magallanes (Amoy Mantan) Pei yu
9. *Fortunella margarita* (Lour.) Swing.	Nagami kumquat
10. *Citrus madurensis* Lour.	Calamansi/calamondin
11. *Citrus reshni* Hort. ex Tan	Cleopatra
12. *Poncirus trifoliata* (L.) Raf.	Beneke trifoliate
13. *Citrus sinensis* (L.) Osb. x *P. trifoliata* (L.) Raf	Carrizo citrange
14. *Citrus paradisi* Macf. x *P. trifoliata* (L.) Raf	Citrumelo

The initial budwoods are taken from Foundation trees and these are increased by propagating on nursery trees in the Budwood Multiplication Blocks. Buds are then harvested from these nursery trees and are released to nurseries for the production of certified plants, which are then sold to citrus growers. For rootstocks seeds, since trifoliate and its hybrids are being demanded by growers, at present, these are being

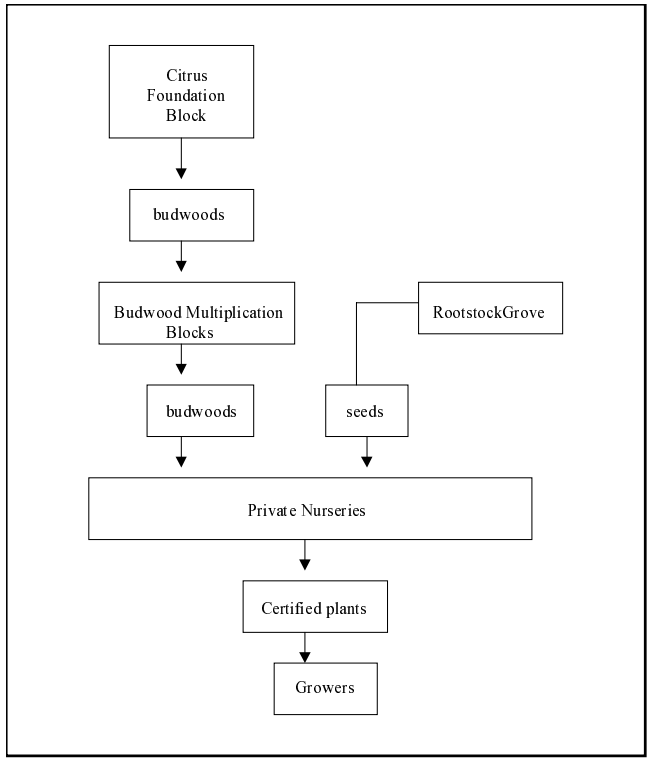


Figure 1. Citrus plant material production scheme.

imported because there are not enough local sources, hence, the need to establish Rootstock groves. To date, there are about 23 private nurseries operating in the Cordillera region. These nurseries obtain their rootstock seedlings and budwoods from BPI. In addition, these nurseries receive technical assistance on proper nursery management and are being monitored to ensure the production of high quality plant materials.

2. Control of insect vectors

During the FAO-UNDP Regional Inter-country Project, studies were done on the biological control of the insect vector of Huanglongbing / greening, *Diaphorina citri* Kuway using traps and parasites (*Tamarixia radiata*) (Mercado *et al*, 1991). Unfortunately, these were discontinued. However, the parasite was found to adopt successfully in the field (Gavarra *et al*. 1990).

Spraying with insecticide is also practiced to control the citrus psylla and aphids. These are timed during the flushing periods because the insect population is high during these time as insects prefer young shoots for feeding and oviposition (Altamirano *et al*, 1976).

3. Eradication and replanting

A system of eradication and replanting is being followed in the case of orchards infected with CTV or Huanglongbing. Trees infected with severe strains of CTV are totally eradicated and replaced. For huanglongbing /

greening infection, however, non-bearing trees (less than four years old) showing symptoms are totally eradicated and replaced, while bearing trees with infection on one or two branches are pruned. However, trees having 50 or 75% infection, depending on the size or age, are totally eradicated and replaced.

4. Cross-protection

The possibility of using mild strains of CTV to control severe stem pitting strains was demonstrated on pummelo (Herradura et al, unpublished). These are important findings and can now be integrated into the planting material production scheme.

Control of other disease

Fungicides are commoly used in the control of most fungal diseases. These include copper-based fungicides for the control of most fungal diseases including bacterial canker, sulfur for the control of powdery mildew, Alfosetyl for root rots / foot rot / gummosis, and captan or benomyl for diseases in the nursery such as damping off.

In nurseries, high budding is practiced to prevent foot rot later in the field. The practice of proper sanitation, care, and maintenance of nursery trees are properly emphasized.

In the field, proper drainage is an important consideration during orchard establishment. After establishment, trees are given proper care and maintenance especially during the early years.

Information dissemination to farmers

There have been aggressive information dissemination campaigns, regular farmers training-seminars, and field exposure programs for growers during the Phil-German Fruit Tree Project. At present, such activities are only done upon request by growers or fruit growers associations.

Conclusion

Despite the problems besetting the industry, the importance of citrus production in Philippine agriculture cannot be discounted because many farmers are still dependent on the industry. In fact, in 1996, the country's citrus-growing areas accounted for more than 30,000 with a production value of P 1.7 B. At present, the citrus industry still thrive in Mindoro and Quezon provinces for the local mandarin and calamansi production; in Davao for pummelo; and in the highlands of Nueva Viscaya particularly in Malabing Valley and in the Cordilleras for the more exotic varieties such as Ponkan mandarins and sweet oranges.

Citrus production still holds promise as a profitable venture. Moreso, because farmers have had a good experience in growing the crop. Hence, the need to pursue the rehabilitation effort.

References

Buitendag, C.H.; von Broembsen A. Living with citrus greening in South Africa. In: Proc. of the 12th International Organization of Citrus Virologists (IOCV) conference, New Delhi, India: 1993. pp. 269-273.

Cortez, R.; Celino, C.S. Distribution of tristeza in Philippine citrus. In: Proc. 4th IOCV conference, Gainesville: Univ. of Florida Press, 1968. pp. 48-51.

Garnsey, S. Virological aspects to an integrated management of vector-borne virus disease of citrus. Food and Fertilizer Technology Center Extension Bulletin 416, 1995.

Garnier, M.; Bove, J.M. Citrus greening disease and the greening bacterium. In: Proc. of the 12th International Organization of Citrus Virologists (IOCV) conference. New Delhi, India: 1993. pp. 212-219.

Jagoueix, S.; Bove, J.M.; Garnier, M. PCR detection of two *Candidatus liberobacter* species associated with greening disease of citrus. Molecular and Cellular probes 10. Academic Press Limited: 1996. pp. 43-50.

Klotz, I.J.; Calavan, F.C.; Weathers, L.G. Virus and virus-like diseases of citrus (A reprint). Division of Agricultural Sciences, University of California. Leaflets 2453, University of California Extension, Riverside.

Ke, Chung. A progress of propagation of citrus disease-free nursery tree in China. In: Proc. of the International Asia Pacific Workshop on integrated citrus health management, Kuala Lumpur, Malaysia: 1991. pp. 52-54.

Martinez, A.; Wallace, J.M. Citrus greening disease in the Philippines. A paper presented at the First International Citrus Symposium; Riverside, California; 1968.

Martines, A.I.; Wallace, J. M. Studies on leaf-mottle-yellows of citrus in the Philippines. In: Proc. of the 4th IOCV Conference, Gainesville: Univ. of Florida Press, 1968. pp. 167-176.

Ochasan, J.M.; Amoy, M.L.; Guerero, M.B.; Verzola, E.A. Occurrence of graft-transmissible diseases of citrus in highlands of Northern Luzon. In: Proc. of the 13th IOCV Conference, People's Republic of China; 1996. pp. 291-296.

Roistacher, C.N.; Moreno, P. The worldwide threat from destructive isolates of citrus tristeza virus - a review. In: Proc. of the 11th IOCV conference, Orlando Florida; 1991. pp. 7-19.

Su, H.J. ; Hung, T.H. Detection of Greening Fastidious Bacteria (GFD) causing citrus greening by Dot Hybridization and Polymerase Chain Reaction (PCR) with DNA probes and primer pairs. (unpublished).

Su, H.J.; Tsai, M.C.; Wu, M.L. Identification and detection of citrus tristeza closterovirus (CTV). (unpublished).

Salibe, A.; Cortez, R.E. Leaf mottling - a serious virus disease of citrus in the Philippines. In: Proc. of the 4th IOCV Conference, Gainesville: Univ. of Florida Press. 1968. pp. 131-136.

Tian, Y.; Ke, S.; Ke, C. Polymerase chain reaction for detection and quantification of *Liberobacter asiaticum,* the bacteria associated with Huanglongbing (greening) of citrus in China In. Proc. of the 13th IOCV Conference People's Republic of China, 1996. pp. 252-257.

Tsai, M.C.; Su, H.J.; Garnsey, S.M. Citrus tristeza virus (CTV) strains and identification by monoclonal antibodies with emphasis on southeast Asia isolates. In: Proc. of the Asia Pacific International Conference on Citriculture, Chiang Mai, Thailand: 1990. pp. 175-180.

Management of Citrus Disease-free Seedlings in Southern Vietnam

Le Thi Thu Hong
Southern Fruit Research Institute
Vietnam

Introduction

About 70% of the total fruit production areas of Vietnam are located in the South. Many of these fruit cultivars have high potential in the local and export market. Citrus is one of the most important of these fruit crops. Most of the citrus production areas are located in the Mekong delta where about 40,000 ha of citrus are grown. The average orchard size in the Mekong Delta is 0.6 ha with an average planting density of 12,000-16,000 plants / ha. However, average yield is only 7.5 t / ha due to the occurrence of diseases. Citrus production in South Vietnam declined between 1960s and early 1980s. It recovered after 1988 when the Vietnamese government shifted to a market economy. From a production area of 7,500 ha in 1990, it increased to about 40,580 ha in 1994 (Table 1).

Table 1. Citrus production in Vietnam.

Region	Existing area (ha)	Major citrus species
Along and between Tien and Hau Rivers (the Mekong Delta)	40,000	Orange, mandarin, pummelo, lime, lemon
Tan trieu Dong nai region	400	Pummelo
Huong thuy-Huong tra Thua thien	300	Pummelo
Huong khe, Huong son (Hatinh) Tuyen hoa (Quang binh) Region	2,500	Phuetrach pummelo, Bu orange (cam bu)
Quy hop Nghia dan-Nghe an Region	3,000	Orange
Citrus Ham yen, Bac quang (tuyen quang) Ha giang Region	500	Orange, mandarin, and some temperate fruit
Yen binh-Doan hung Yen son Region	600	Pummelo, orange, mandarin

Source: National Institute of Agricultural Planning and Projection 1994

Citrus is grown in many provinces of Vietnam. Cantho (in the Mekong Delta) has the largest with a production area of 15,000 ha. Other citrus- growing provinces are Tien giang, Vinh long, Bentre, Tra vinh, Hoa binh, Nghe an, and Lang son.

Disease infection was one of the major factors which caused the declined in citrus production in the South. Among the citrus virus and virus-like diseases existing in the South, Huanglongbing (HLB) and citrus tristeza virus (CTV) were the most destructive. HLB is widespread throughout Vietnam, especially in areas where *Diaphorina citri,* the psyllid vector of the disease is present (Trung 1991). Aside from HLB, other existing diseases in the south are exocortis, cristacortis, phythothora gummosis, Rio Grande gummosis and citrus canker (Bove *et al*., 1995).

In order to contribute in the rehabilitation of the citrus industry in Southern Vietnam, SOFRI initiated a R and D program in 1995 with the following components:

1. Survey on the occurrence and impact of HLB and CTV infection and the existing nursery management practices in Southern Vietnam
2. Eradication of infested trees
3. Production and distribution of disease-free planting materials
4. Control and management of insect vectors
5. Demonstration of the Integrated Citrus Orchard Management

This paper presented only some aspects of the program related to the management of citrus disease-free seedlings in Southern Vietnam. The emphasis is on the contribution of SOFRI in the development of the citrus industry.

Incidence of HLB and Tristeza on citrus

Disease. Surveys conducted in 1995 on the Huanglongbin disease showed that HLB infested all citrus-growing regions in South Vietnam. Particularly in the Mekong delta, contributory factors in the spread of the disease are high planting density, high psyllid population, and the use of infected seedlings. It was observed that 4-7- year-old orchards were the ones severely damaged. The survey also showed that king mandarin and sweet mandarin were the most susceptible citrus varieties.

The study revealed that the *Diaphorina citri* (HLB vector) and aphid (tristeza vector) population was high during the month of January. It was also recorded that of the three natural enemies of *Diaphorina citri, Daphorencytus aligahensis* and *Tamarixia radiata* were effective with 15.3-26% parasitism (Hong *et al*., 1995).

More than 180 samples taken from different citrus species in almost all of the provinces in the Mekong Delta were infected with tristeza based on the positive reactions when subjected to the ELISA method. However, some samples of pummelo from Cantho, Vinh long, and Bentre were found negative of the disease (Hong and Trung 1997). About 33.6% of the total area surveyed has 15-30% infection in Long hoa and Cantho (Hong *et al.* 1997). The vein clearing symptom of tristeza is present in almost all of lime plantation. ELISA was used on the infected trees to confirm the existence of the mild strain of tristeza virus. Researchers also hypothesized that the natural mild strain tristeza serves as natural cross protection against the severe strains of stem pitting and dwarfing. However, these mild strain infected trees could serve as sources of infestation for newly established citrus orchard.

Effects of the HLB infestations. The survey was done in Cantho province, the largest citrus-growing province in the country. A total of 90 questionnaires were used to interview citrus growers in the villages of Long tuyen (10), Long hoa (10), My khanh (10), Thanh xuan (10), Dong thanh (10), Dong phuoc (10),

Tan phu thanh (10),and Thanh hoa (20). Total area surveyed was 45 ha, with an average of 0.5 ha / household. The result of these investigation showed that about 70% of the orchards were 5-7 years old. Symptoms appeared when the plants were about 4-5 years. About 66.7% of farm households had severely infested orchards while 33.3% had medium disease index. When asked about their knowledge on Huanglongbin (Vang la Greening) only 20% knew the exact causal pathogen. Almost all of the respondents voiced out that they would be resorting to the cutting down of trees due to the projected low harvest. However, about 36% wanted to replant (Table 2 and Table 3).

An evaluation of the losses due to Huanglongbin in Cantho revealed that sweet orange had suffered most losses followed by king mandarin and sweet mandarin. The least affected was pummelo and the others citrus varieties. However, accurate evaluation of the damages caused by HLB was difficult as other factors were unknown. Early infection of HLB caused highest losses. In summary, 66.7% of the total orchards surveyed in the Mekong Delta were severely infected. Before infection, the average yield of sweet orange was 1945 t / ha; King orange, 16.68 t / ha and mandarin, 12.10 t / ha. After infection,

Table 2. Cantho Farmers' knowledge on the causal agent of Huanglongbin (Vang la Greening) (1997).

Percentage of interviewed growers (%)	Cause of HLB infestation
20	Bacteria Vang la greening
70	Environmental problem: Abnormal climate, soil problem, water logging
10	Do not know the reason / no idea about this

Table 3. Cantho farmers' preferred fruit crop to replace infected citrus (1997).

Fruit tree for replanting	Percentage of citrus growers(%)
Citrus	36
Others like mango, longan, guava, jujube and durian	44
Not yet decided	20

these were decreased to 3.78 t / ha, 3.19 t / ha and 2.22 t / ha, respectively. And in some areas, the tree performance was very poor that nothing was harvested (Table 4 and Table 5).

Table 4. Average yield losses of King mandarin, sweet orange, and sweet mandarin.

Sweet Orange (t/ha)			King mandarin (t/ha)			Sweet mandarin (t/ha)		
Before symptom	After symptom (at present)	Yield loss	Before symptom	After symptom	Yield lost	Before symptom	After symptom	Yield loss
19.45	3.78	-15.67	16.68	3.19	-13.49	12.10	2.22	-9.88

Table 5. Average yield of King mandarin, sweet orange, and sweet mandarin in Cantho (1997).

Sweet orange (t/ha)			King mandarin (t/ha)			Sweet mandarin (t/ha)		
Before symptom	After symptom	Yield loss	Before symptom	After symptom	Yield lost	Before symptom	After symptom	Yield loss
19.45	5.25	-14.20	16.68	4.84	-11.84	12.10	3.12	-8.98

Citrus nursery management in private nursery sector

About 7,000-9,000 citrus nurserymen in Bentre are believed to be propagating planting materials infected with Huanglongbin, citrus canker, and severe strains of tristeza (Philippe *et al*., 1995). However, some private nurserymen in Cantho province realized that propagating grafted seedling may lead to further infection, thus, they have started producing through seeds and selected the nucellar seedlings. These seedlings are then marcotted to improve the root system for water logging tolerance and other characteristics (Hong and Trung, 1996).

It was noted that citrus nurseries have poor quality of irrigation water and sanitation. Marcotting was still the most commonly used propagation method for mandarin and pummelo. Sweet orange (cam mat) rootstock were found susceptible to root rot and water logging.

The cheap cost of producing a citrus seedling indicated low input requirement but the net return was relatively high. Moreover, the frequent need for replanting of seedlings due to very short life created more demand for seedlings (Table 7).

Production and distribution of disease-free planting materials

The application of shoot tip grafting and disease indexing, including molecular biological indexing of the SOFRI citrus disease-free seedlings production program was successful (Hong *et al*., 1997).

SOFRI, with the technical cooperation of the Centre de Cooperation Internationale en Recherche Agronomique pour le Development-Departement des Productions Fruitieres et Horticoles (CIRAD-FLHOR), Food & Fertilizer Technology Center (FFTC), and the

Table 6. Major insect pest and diseases recorded in private nurseries in Cantho (1995)

Insects	Scientific name	Percentage	Percentage of damaged plants	No. of leaves observed	Number of leaves damaged	Percentage
Leaf miner Leaf cutter Aphids	*Phyllocnictis citrella* *Papillio* spp *Toxoptera* spp	100 70 20	62.23 13.80 1.24	1875 13.80 520	551 123 188	29.39 12.06 23.50
Diseases	Pathogen			Percent infested orchard (%)	Percent infested trees (%)	Disease index (%)
Canker Scab Root rot Dry Root rot	*X. campestris pv. citri* *Ellisinos faweettii* *Phytophthora* spp *Fusarium* spp			90 60 30 20	21.00 25.50 - -	54.50 63.40 - -

Nutrient and irrigation management in citrus nursery

Table 7. Nutrition and irrigation management in citrus nursery in Cantho (1995).

	Medium	Fertilizer	Irrigation
Rootstock	Ash, coconut, fiber, sand (1:1:1)	- 1 month after growing - 1 spoon / 10 L of water	Keep constantly moist
Land prepared for grafting	Any land or soil available	1 spoon urea / month	
Grafted block	"	"	Stop watering 1-3 days after grafting
Marcotting preparation	mud coconut finer	Nil	

Table 8. Economic efficiency of citrus nursery production in Cantho (1995).

Techniques in propagation	Input (US$/plant)	Sale (US$/plant)	Net return (US$/plant)	Number of seedlings
Grafting Marcotting Seed sowing	0.0281 0.0145 0.0250	0.2280 0.1462 1,085	0.1999 0.1322 0.0835	15,558 562

Australian Center for International Agricultural Research (ACIAR) and the financial support from the National Research Program (Ministry of Science Technology and Environment), Ministry Program (Ministry of Agriculture Rural and Development), Extension program (Department of Agriculture Extension of MARD), the provincial collaborations and R and D institutions has been studying and applying shoot tip grafting and disease indexing to produce the disease-free mother trees.

Shoot tip grafting was conducted through a modified technique under the guidance of CIRAD-FLHOR and FFTC (Taiwan University).

Molecular indexing through PCR was used to detect *Liberobacter asiaticum,* pathogen of Huanglongbin with the guidance and protocol of Prof. Hong Ji Su (Taiwan University), Dr. Granier (Institut National de Recherche d' Agriculture (INRA-France), Dr. Barkley and Dr. Deborah (Elizabeth Macarthur Agricultural Institute (EMAI-Australia). Canker (*Xanthomonas* pv *citri*) Exocortis viroid was also detected through PCR techniques with protocol from the Division of Plant Pathology of EMAI.

So far, SOFRI has produced about 28,000 healthy plants to supply the requirement of demonstration plots and yield trials. In addition to disease-free mother trees produced from native cultivars, the evaluation and selection for the most adapted cultivars among 30 promising varieties imported from Corse (France) could also provide a considerable number of healthy budwoods (Philippe *et. al*, 1997.)

Among the 85 STG trees, 53 have been indexed and 52 / 53 are healthy. The cost of producing a disease-free seedling was US$2. The seedlings undergo rapid multiplication for further application (Table 8).

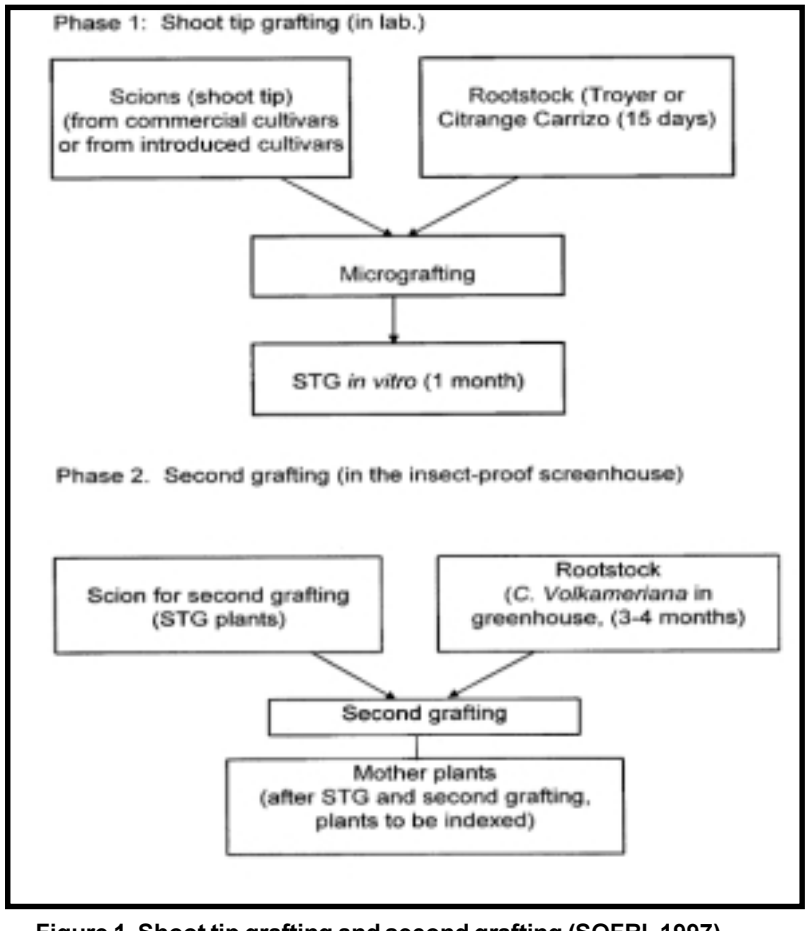


Figure 1. Shoot tip grafting and second grafting (SOFRI, 1997).

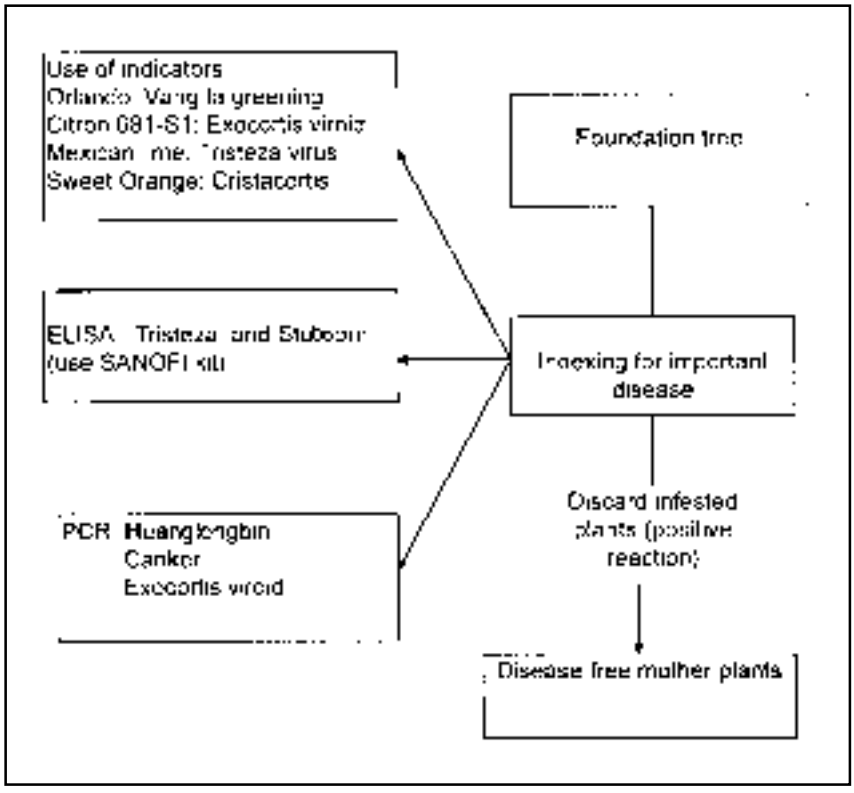


Figure 2. Disease indexing after shoot tip grafting (SOFRI 1997).

Demonstration of citrus integrated orchard management

The demonstration of Integrated Citrus Orchard Management was conducted in a total of 10 ha in selected provinces in the Mekong Delta. In 1996-1997, the demonstration of growing healthy citrus seedlings was established in six provinces. A total of 5,166 disease-free seedlings were grown on Volkameriana or trofoliate rootstock. These include 448 Duong mandarin seedlings (Cantho, dong thap and Soc trang sites), 624 tieu mandarin seedlings (Cantho, Dong thap, and Soctrang sites), 3,504 king mandarin plants (Cantho, dong thap, Soctrang, Tien giang and Bwentre sites), and 590 sweet orange plants (Cantho Tien giang and Bentre sites).

In 1997-1998, the program continued to establish more demonstration orchards in six provinces producing a total of 5,755 healthy plants on Volkameriana rootstock. The seedlings include 960 Duong mandarin (in Cantho, Tra vinh and Tien giang sites), 880 Tieu mandarin (in Cantho, Vinh long, Tien giang, and Bentre sites) 240 sweet orange (Canthe), 525 nam roi pummelo plants (in Cantho and Tein giang sites).

Some of the lessons learned from the demonstration program and drawn from the recent meetings with farmers / cooperators and extensionists are the following:

- King mandarin grafted on Volkameriana gave good yield and quality as early as 20 months after planting. However, the vector could not be effectively controlled and this was observed in Soctrang site (100%), in Bentre (52%), in Cantho (50%) and 16% in Tien giang (16%).
- The re-infection of Huanglongbin occurred 100% at Soctrang sites as a sequence of the transmission of the high psyllid population, 50% at Cantho site, 23% at Bentre site and 16% at Tiengiang site. In general, the best was Tiengiang site where the infested trees had been removed very long time before the reestablishment of new plantings.

Conclusion

Huanglong bin disease has become a major threat to citrus production in Southern Vietnam particularly Mekong Delta which is the largest citrus-growing areas.

Private citrus nurseries play an important role in seedling production. However, this citrus nurseries are faced with problems such as marcotting technique lead to Rio grand gummosis infection of pummelo trees in Vinh long

Table 9. Number of disease-free mother trees produced per cultivar (SOFRI 1995-1998).

Sr./No.	Citrus cultivar	No. of plants	Identify entries
1	King Mandarin (Cam sanh)	14	CS-TG (2,21,5,43,29,34,45,15, 7,13,8,9,119,111)
2	Sweet Orange (Cam mat)	13	CM-STG (14,28,16,3,20,70,71,72,50, 22,26,17,10)
3	Tieu Mandarin (Quyt Tieu)	9	QT-STG (35,52,32,33,53,37,47,69, 40, 56,121)
4	Duong Mandarin (Quyt Duong)	4	QD-STG (47,69,120,159)
5	Nam Roi Pummelo (Buio duong la cam)	4	B5R-STG (4,24,23,94)
6	La cam sweet Pummelo (Buoi duong la cam)	1	BDX-STG (42,74,75)
7	Da xanh Pummelo (Buoi da xanh)	1	CT-STG (42,74,75)
8	Tau Lemon (Chan tau)	4	CT-STG (42,74,75)
9	Giay Lime (Chanh Giay)	2	CG-STG (59,67)

provinces, virus and virus-like pathogen infestation and propagation from infested mother trees. Hence, the following recommendations are given:

- There is an urgent need to give more focus on extension programs for specific target groups such as growers, nursery men and extensionists; and
- The sanitation measures and legislation for the establishment of a National Citrus Certification Scheme must be enforced to successfully reorganize citrus production in the region and to enhance a national rehabilitation program.

References

Aubert, B; Hong, L.T.T. Prevention of some citrus infectious diseases to increase the high efficiency for citrus growers in Vietnam. Agricultural Publish House: Ho Chi Minh, 1996. (Vietnamese).

Bove, J.M; Chau, N.M; Trung, H.M.; Bourdeau, J.; Garnier, M. Huang longbin in Vietnam: detection of *Liberobacter asiaticum* by DNA hybridization of 16S ribosomal DNA. In: Proceedings of the 13th Conference of the International Organization of Citrus Virologists. California USA: University of California, 1995. pp. 258-266.

Hong, L.T.T.; Trung, H.M. About the citrus nursery management of Chau thanh, Cantho. Agricultural Publishing House: Hanoi. 1996 (Vietnamese).

Hong, L.T.T.; Trung, H.M. Result of the survey on citrus tristeza in the Mekong Delta. Journal of Science, Technology and Economic Management: p. 259, 1997. (Vietnamese).

Hong, L.T.T.; Hoa, N.V.; Duc, H.T.; Nuong, L.T.M.; Thanh, H.V. Preliminary results on the observation of virus and virus-like diseases of citrus in the Mekong Delta region of Vietnam. In: Proceedings of the 13th Conference of the International Organization of Citrus Virologists. California USA: University of California, 1995. pp. 339-400.

Hong, L.T.T.; Trung, H.M. Investigation on Vang la greening in the Mekong Delta. Agricultural Publishing House: Hanoi, 1996. (Vietnamese).

Hong, L.T.T.; Chau, N.M.; Thu, T.P.A.; Xuyen, T.T. Preliminary result on the production of disease-free plants at Long dinh. Fruit Research Center Journal of Science, Technology and Economic Management: pp. 245, 1997 (Vietnamese).

Hong, L.T.T.; Hoa, N.V.; Xuyen, T.T. Preliminary result on the study on tristeza on lime. Journal of Science, Technology and Economic Management: pp. 258, 1997.

Phililppe, C.V.; Hong, L.T.T.; Thu, T.P.A.; Chau, N.M. Supply of elite plants to the citrus industry in the Mekong Delta. In: Proceedings of the 5th International Congress of Citrus Nurserymen, Montpellier, France: 1997.

Trung, H.M. Surverys on the citrus greening and its control by IPM in Vietnam. Rehabilitation of citrus industry in the Asia Pacific Region: Kuala Lumpur, Malaysia, 1991. pp. 26-35.

Management of Citrus Disease-free Seedlings in Northern Vietnam

Ha Minh Trung
Ngo Vinh Vien
Ngo Dinh Phu
National Institute of Plant Protection
Hanoi, Vietnam

Abstract

Citrus is an important fruit crop in Vietnam. However, citrus production in the country is currently declining due to citrus greening disease.

A program for disease-free seedlings production and implementation of our integrated pest management for Asian psyllid has been initiated to rehabilitate and develop the citrus industry in Vietnam. The program use micrografting technique to produce disease-free mother trees. The ratio of successfully micrografted seedling had an average of 46.2%. About 55 disease-free seedlings of Canh and Xa Doai orange cultivars, and Dien pummelo were kept in an insect-proof screenhouse at the National Institute of Plant Protection. These were the first stock of disease-free budwoods to be provided to the disease-free nursery system in Northern Vietnam.

Introduction

Citrus is one of the major fruit crops in Vietnam. Vietnam was a citrus exporter to Eastern European countries during the 1970s. However, in the past decades, the citrus industry experienced a decline in production due to the greening disease. Vietnam is currently turning from a citrus exporter to an importer of citrus from China and Australia. Despite this, there is a great potential for citrus development in the country.

At present, the total production area for citrus is estimated at about 70,000 ha with an annual production of approximately 700,000 t. Part of the government's program for agricultural diversification is to expand the production area under citrus up to 100,000 ha. The new citrus plantations shall be mainly located in the northern highland and mountainous regions where plenty of land resource is available and the climate is suitable for citrus production.

A research and extension program for the production of disease-free citrus seedlings and integrated pest management of Asian psyllid *Diaphorina citri* has been initiated by the government in order to rehabilitate and develop the citrus industry.

The Food and Fertilizer Technology Center (FFTC) has provided technical and partial financial support to the above program with the aim of establishing pathogen-free foundation stocks and the production of healthy citrus seedlings. This report will present the works undertaken for the future plan of the citrus industry.

Citrus Industry Development Program in Vietnam

The program involves collection of disease-free mother trees and the production of disease-free seedlings. It has the following objectives:

- To establish a collection of disease-free mother trees by micrografting technique;
- To produce grafting budwoods (scions) maintained under an insect-free screenhouse; and
- To produce disease-free seedlings in screen nurseries.

Citrus mother trees were taken from traditional Vietnamese citrus cultivars and micrografted. Micrografting is a technique provided by Taiwan University and a number of other research institutions. This technique was improved and applied in the National Institute of Plant Protection (please see Figure 1).

Phytosanitary control under the screenhouse and the nursery was implemented as described in Figure 2. However, only indexing technique was used for greening and ELISA test for Tristeza due to lack of equipment and facilities.

Five micrografting were conducted on two orange cultivars (Canh and Xa Doai) and one pummelo cultivar (Dien) (Table 1).

The average percent of successful grafts were 46.2%. The first 55 disease-free seedlings will provide the required (55 x 500) disease-free budwoods for 1999. This is to ensure the operation of the disease-free citrus nursery system in the country.

Table 1. Percentage of successfully grafted seedlings by shoot tip grafting in 1998.

	Cultivar of shoot tip	Number of micrografts	Number of successful grafts	% of successful grafts
1	Dien pummelo	21	5	23.8
2	Canh orange	21	8	38.1
3	Canh orange	38	15	39.4
4	Xao Doai orange	17	11	64.7
5	Xao Doai orange	22	16	42.7
	Total / Average	119	55	46.2

The Citrus Disease-free Nursery System

The disease-free citrus nursery system was established in conformity with the Taiwanese model. It consists of the following:

- The National Foundation Nursery where disease-free rootstocks and mother trees are kept to produce disease-free budwods;
- The National Budwoods / Scions Nursery where mother trees are kept to produce disease-free budwoods for distribution to local nurseries; and
- The Local Nursery where disease-free rootstocks are grown and disease-free budwoods are grafted to produce disease-free seedlings for local citrus growers.

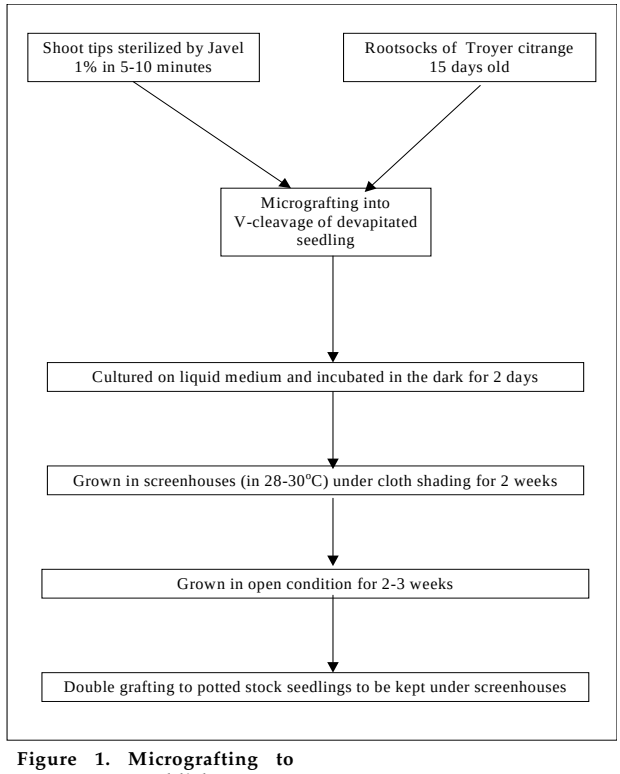


Figure 1. Micrografting to establish pathogen-free citrus foundation stocks.

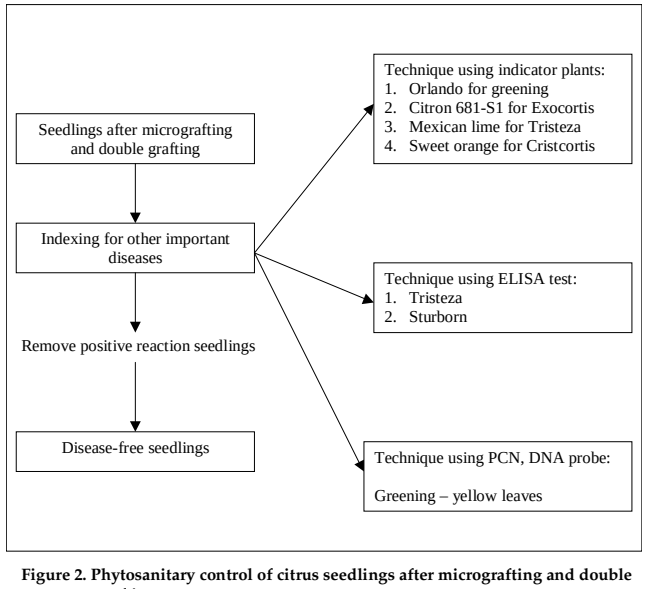


Figure 2. Phytosanitary control of citrus seedlings after micrografting and double grafting.

In Northern Vietnam, an insect-proofed screenhouse measuring about 250 sq.m. is located at the national foundation nursery in the National Institute of Plant Protection Hanoi. It was established with support from FFTC.

The first disease-free citrus seedlings have been produced and kept in this screenhouse. While awaiting for additional number of mother trees, a part of the screenhouse is being used to produce disease-free budwoods / scions for the establishment of models for citrus rehabilitation and development in Vietnam.

References

Aubert B. Integrated citrus health management in South Asia. In: Proceedings of the 6th International Asia Pacific Workshop, Kuala Lumpur, 29-30 June 1991.

Ha Minh Trung. Surveys on citrus greening and its control by IPM in Vietnam. In: Proceedings of the 6th International Asia Pacific Workshop on Integrated Citrus Health Management, Kuala Lumpur, Malaysia, 24-30. June 1991.

Su H.J., Chen, C.N. Implementation of IPM on citrus virus and greening (Likubin) diseases. FFTC supplement No. 1, 1991. pp. 3-11.

Establishment of Disease-free Foundation and Nursery for Controlling Greening Disease and Citrus Tristeza Virus: A Sarawak Experience

Teo Chan Hock
Agricultural Research Centre
Department of Agriculture
Malaysia

Summary

Citrus greening disease was detected in Sarawak in the mid 1980s. The citrus industry was badly affected. In an effort to revive citrus production in the state, the Department of Agriculture – Sarawak started a program to produce disease-free citrus planting materials. Shoot tip grafting, rapid propagation, and disease detection techniques were obtained from the National Taiwan University. Bud eyes and bud-grafted plants were being produced by the research branch of the department and a farmer organization, respectively. However, production has not been able to meet the target set by the department due to some problems encountered. Efforts were made to increase the production.

Introduction

In Sarawak, a Malaysian state situated in the Borneo island, citrus production is done in small scale. The produce is mainly for local consumption. The mandarin variety Langkat (*Citrus reticulata* Blanco), is most commonly planted by the farmers while sweet orange (*C. sinensis*(L.) Osb.), Pummelo (*C. grandis* (L.) Osb), lemon (*C. limon* (L.) Brum.f.), lime (C. *aurantifolia* (Christm.) Swing) and Kasturi line (*Calamondin madurensis* Lour) are planted only in small numbers. In the early 80s, the Department of Agriculture – Sarawak provided subsidy scheme to farmers for fruit tree planting to encourage them to plant more Langkat mandarin. Marcotted plants were imported from outside the state to fulfill requirement for the planting materials. The average of citrus farms increased to about 2000 ha with 1,200 ha located in Samarahan Division.

However, even before the drive to increase citrus production, diseases have already been observed in the state. In the 70s, citrus tristeza virus disease was already observed to exist. The disease did not cause serious damage to the citrus plants though even as disease symptoms such as stem pitting were obvious on Langkat and Kasturi lime. CTV was not as threatening as the greening disease.

In the mid 80s, greening disease started to appear in some citrus farms. By early 90s, majority of the farms were infected. Production decreased and farmers abandoned the tress and planted other crops. Citrus fruits had to be imported from overseas to meet the local consumption.

Since then, the Department of Agriculture has decided to revive the citrus production in the state. Production of disease-free planting materials was initiated in the research division of the department along with research studies on the control of the insect vector *Diaphorina citri* were implemented.

Setting up of production facilities in the research center

In order to produce GO- and CTV- free citrus planting materials, research officers studied at National Taiwan University in Taiwan in 1994 to learn shoot tip grafting, rapid propagating techniques and the setting up of needed facilities. In addition, techniques for the detection of GO and CTV were also acquired.

In 1995, two citrus foundations were either built or modified for the production of disease-free citrus planting materials. Two more were built in 1996. Seeds of Swingle citrumelo, Carrizo citrange and Cleopetra mandarin were purchased from the US and Australia. Due to lack of equipment in the laboratory for GO and CTV detection, plants produced from shoot tip grafting as well as those grafted with bud-eyes collected from healthy looking plants in farmers' fields were sent to Professor Su Hong-Hi of the National Taiwan University for the detection of both diseases. Only the disease-free plants were kept as foundation stocks from which bud-eyes were collected to produce mother plants. About 2,000 mother plants were kept in the research center for bud-eyes collection. The research center produced 6,000 plants from 1995 to 1996 and distributed to farmers for field planting.

In 1997, the department acquired thermal cycler, gel electrophoresis equipment and the necessary chemicals for production purposes. The polymerase chain reaction (PCR) and gel electrophoresis techniques were learned from Professor Su who also provided the primers for the PCR. The research center is now able to carry out the greening disease detection work.

Production of citrus planting materials by farmer organization

The Sarawak Department of Agriculture plans to help citrus farmers in Samarahan Division to replant 1,200 ha of land with citrus by the year 2000. A total of 326,400 healthy citrus plants are needed for this purpose. In addition, more plants is also required for new planting in other divisions in the state. The research center's main task is to produce disease-free bud-eyes rather than the planting materials. The Farmer Organization (FO) is assigned to produce bud-grafted planting materials in large quantity. The department will then purchase the plants from the FO at an agreed price and issue them to farmers who will participate in the citrus planting scheme.

In late 1996, the Farmer Organization in Serian, Samarahan Division constructed two screenhouses for seedlings and rootstocks establishment. To date, six additional screenhouses had been built. Initially, two workers were employed to carry out the grafting work.

Starting in January 1997, the research center provided bud-eyes to the farmer organization. A total of 70,000 bud-eyes was supplied for that year. The Farmer Organization managed to supply 26,384 plants to the department during the year. In the early 1998, the number of screenhouse staff increased to four in an attempt to speed up production. Up until July 1998, 99,000 bud-eyes were supplied to FO Serian. FO sold 13,986 plants to the department. Presently,

there are 22,000 bud-grafted plants at various growth stages still being kept in the nursery. By the end of 1998, FO would have to supply another 40,000 plants to produce a total of 80,370 plants for 1997-98 (Table 1).

Table 1. Percentage of successfully grafted seedlings by shoot tip grafting in 1998.

	Cultivar of shoot tip	Number of micrografts	Number of successful grafts	% of successful grafts
1	Dien pummelo	21	5	23.8
2	Canh orange	21	8	38.1
3	Canh orange	38	15	39.4
4	Xao Doai orange	17	11	64.7
5	Xao Doai orange	22	16	42.7
	Total / Average	119	55	46.2

Production associated with planting material production

In the production of planting materials, a number of problems were encountered:

1. **Bud-eye production.** Due to slow growth of the mother plants inside screenhouses, the production of bud-eyes was initially very slow. Possible reasons for slow growth are screenhouse temperature (above 35°C during the day), shading effect of the roofing and the restricted root growth inside pots. A one-year-old plant produced about 150 bud-eyes / year. This could not meet the requirement of the FO. To solve the problem, it was decided to plant disease-free plants in the field at the research center. These plants were and still are subjected to heavy spraying of insecticides at weekly intervals to prevent psyllids and aphids infection. The plants were subjected to regular checking for the diseases. It is done by observation for disease symptoms at monthly intervals and laboratory test for the pathogens whenever disease symptoms are observed. Bud-eyes are now collected from these plants for grafting in FO nursery. This is a stop gap measure with the aim of producing enough planting materials to meet the demand. Meanwhile, another 1000 bud-grafted plants are being produced and maintained inside screenhouses as mother plants. Future bud-eye collection will be from these plants rather than those planted in the field. More screenhouses are required to maintain the plants.
2. **Grafting skill of the budders.** The budders working in FO Serian nursery practice conventional grafting work. They prefer to do grafting on rootstocks which are at least six months old (bigger stem) and make use of bigger bud-eyes from field plants rather than those growing under the sceenhouse condition. Admittedly, this is not in accordance with the rapid propagation method advocated by Professor Su. It inevitably slows down the process of planting material production because longer time is required for the bud-eyes to attain the desirable size. The rootstocks will have to be older to reach the size required. Effort are being made to train the budders to adopt new techniques.

   The budders use chip grafting (single bud-eye) and side veneer grafting (two or more bud-eyes) techniques in their grafting work. Side veneer grafting is employed if the bud-eyes are younger. As such, more bud-eyes

are required. Out of the 99,000 bud-eyes collected so far in 1998, 52,297 plants were grafted. The expected success rate of grafting is 60 – 70%, depending on the skill of individual budder. These put a lot of pressure on the bud-eye production.

3. **Rootstocks.** The rootstocks used for grafting by FO Serian are Carrizo citrange and Cleopetra mandarin. With Carrizo citrange, it takes at least eight months before a grafted plant is ready for field planting, while with Cleopetra mandarin, it takes 10 months. The latter grows slower and the bud-eye grafted on it also takes longer time to sprout and produce enough leaves.
4. **Screenhouse facilities.** The FO Serian nursery started with two screenhouses. They were inadequate to house the large number of plants need to be produced. Nursery beds for seed germination were also lacking. More screenhouses were being built in 1998. At present, a total of eight screenhouses are available in the said nursery to boost the production.

The future direction in the production of citrus planting materials

Malaysia has been hit by the economic downturn since July 1997. The Malaysian government has formulated a food production policy to increase food production as well as to reduce the import of food items. Sarawak imported RM 1.42 million (US$ 0.37 million) worth of citrus fruits and RM 4.80 million (US S 1.26 million) of citrus juices in 1997 (Table 2).

To reduce the importation on citrus, the state government of Sarawak decided to increase citrus production to produce enough citrus fruit for both local consumption and downstream activity such as juice processing. The state government also aims to make Sarawak a net exporter of citrus in the future. In order to achieve this, bigger acreage of citrus planting is required. This means that the production of citrus planting materials has to be increased correspondingly.

Thus, the immediate target of the FO nursery in Serian is to meet the requirement to replant 1,200 ha of citrus gardens in Samarahan Division by the year 2000. This requires a total of 326,400 plants. A balance of 246,000 plants are still to be produced for the years 1999 and 2000. More mother plants need to be established for bud-eye production. The FO nursery will also have to speed up production of the planting materials.

Table 2. Statistics on the importation of citrus fruits and citrus-related juices to Sarawak in 1997.

Product	Ton	RM *	US$ **
Citrus fruits	1,247	1,416,159	372,673
Citrus related juices	14,499	4,802,204	1,263,737

* RM = Ringgit Malaysia
** US$ 1.00 = RM 3.80 (Currency exchange rate)Malaysia

Source: Agriculture Statistics of Sarawak 1997 (to be published).

Status of Banana and Citrus Viral Diseases in Indonesia

A. Nurhadi
L. Setyobudi
Research Institute for Fruits
Solok, Indonesia

Abstract

Banana and citrus are important commercial fruit crops in Indonesia with priorities set in agribusiness development. The demand for domestic and international markets continue to increase during the last five years, however, productivity of both commodities is relatively low compared to banana and citrus producing countries in Southeast Asia. Banana and citrus are mostly grown in backyard or small holder farms using limited production input and technology. Five viral diseases on citrus, and three viral diseases on banana have been confirmed prevalent in Indonesia. CVPD, tristeza, BBTV, and CMV are recognized as the most serious threats to their respective crops. Level of incidence and severity varies between varieties and regions depending upon the agro-ecological zones. This paper describes geographical distribution, potential impact, and indexing method against the diseases of banana and citrus in Indonesia. Current status of disease control programs are briefly discussed.

Introduction

Banana and citrus are important fruits grown in Indonesia. Both dominate 42.35% of annual per capita fruit's consumption of urban and rural folks (Setyobudi and Purnomo, 1998). Banana plays an important role in the food security of rural villages which shares 47.2% of the country's total annual fruits consumption.

Banana and citrus are primarily grown in backyards or small holder's orchards using limited input production and technology. Consequently, the productivity is low. However, since the last decade, medium and large scale banana and citrus orchards began to expand in some provinces in Indonesia due to significant increase in domestic and export market demands.

Banana

Since 1991, some agribusiness companies have started the establishment of large banana estate for export to South Korea and Japan. These are PT. Abana in Riau, PT. Multi Agro in Lampung, PT. Smart in Halmahera, PT. 23 in Sulawesi, PT. Hasfarm in Irian Jaya and PT. Sinar Mas I. Most of those companies grow Cavendish cultivars.

Banana production area has been decreasing annually at the rate of 1.3%, however, production has increased at 4.8% per year. This shows that yield has increased at 6.1% per year. More than 60% of bananas are produced in Java. The largest share

was observed in West Java, which accounted for 23.0% of the national production, followed by East Java and Central Java at 17.8 and 16.8%, respectively. Other provinces contributed at least 10% each. This geographical pattern is proportional to the distribution of population in the corresponding province.

Pisang Ambon Kuning and Ambon Hijau were the major cultivars planted before epidemic incidence of wilt disease caused by *Fusarium oxysporum* f. sp. cubense occurred affecting the production of the cultivars. Pisang Kepok is recently the preference of farmers because it is easy to grow, has many uses and tolerant to Foc disease but very susceptible to bacterial wilt or blood disease.

Citrus

Between 1983-1995, total production of citrus increased, however, productivity was declining, primarily due to CVPD (greening-like disease). The decline in productivity was sharper because of unavailability of disease-free planting materials, lack of technology on integrated pest and disease control, and ineffective technology transfer. Siem was a dominant citrus variety followed by Mandarin. Siem has poor quality but preferred by farmers because it is easy to grow and high yielding. In 1986, 57% of the harvested area was devoted to Siem and contributed 62.2% of total citrus production of 574.322 tons. Many varieties of Mandarin contributed 15.7% of the harvest and accounted for 22.2% of the production. Majority of Siem growing areas are in Riau province, followed by West and Central Java. The highest average productivity of Siem was in West Kalimantan (17 t / ha) followed by East Java (11.12 t / ha), and West Java (8.61 t / ha).

Banana and citrus virus diseases in Indonesia

Banana

Three virus diseases on banana have been confirmed prevalent in Indonesia (Table 1). BBTV is recognized as the most serious threat based on current economic impact, geographical distribution and effect on commercial cultivars (Table 2), while CMV and BSV are rarely found in farmers' orchards. Incidence of BBTV and CMV particularly occurred at banana estate where tissue-cultured plant materials have been introduced. Level of incidence and severity varies between varieties and regions depending upon the agro-ecological zones. The banana viral diseases discussed below focused on major diseases causing substantial losses and resulting to serious economic effects.

Banana Bunchy Top Virus (BBTV). BBTV is considered the most serious viral disease affecting banana and plantain in Indonesia. BBTV was first reported to occur in West Java and in Bali (Sulyo, 1978). It was observed in Sabah and Papua New Guinea since 1930. In Kalimantan, Sulawesi and Irian Jaya, BBTV was found earlier. BBTV recently reached epidemic level in most banana growing province of Lampung and Central Java. Although production losses have not been quantified, the significant economic impact have been affecting the farmers' welfare.

Table 1. Geographical distribution of banana and citrus virus diseases in Indonesia by province.

	Banana			Citrus				
Provinces	**BBTV**	**CMV**	**BSV**	**CVPD**	**CTV**	**CEV**	**CPV**	**CVEV**
Aceh					+			
North Sumatera				+	+			
Riau	+			+	+			
West Sumatera	+	+	+	+	+			+
South Sumatera				+	+			
Lampung	+++	++	+		+			
West Java	+++	++	+	++	+			
Central Java	++	+		+	+	+	+	
Jogyakarta	+			+	+			
East Java				++	+-	+	+	+
Bali	+			+++	++	+	+	
West Nusa Tenggara				++	+			
East Nusa Tenggara					++			
West Kalimantan					+			
South Sulawesi				+	+			

+++ Wide : found in more than 75 % of growing regions in the province
++ Moderate : found in 25 - 75 % of growing regions in the province
+ Localized : found in less than 25 % of growing regions in the province

BBTV : Banana Bunchy Top Virus
CMV : Cucumber Mosaic Virus CPV
: Citrus Psorosis Virus BSV :
Banana Streak Virus
CVPD : Citrus Vein Phloem Degeneration
CTV : Citrus Tristeza Virus
CEV : Citrus Exocortis Viroid
CVEV : Citrus Enation - Woody Gall

Occurrence of BBTV in Indonesia is different with that in India as reported by Singh (1980) where incidence of BBTV is not noticed in areas with high temperature. The incidence of BBTV in Indonesia was more related to the agronomic practices of using sucker.

BBTV is caused by isometric particles and multi-component ssDNA genome, with round particles of 18-19 nm and, is reported to be similar to those in Australia, Hawaii, and Tonga (Dietsgen and Thomas 1991). At early stage of development, dwarfing with dark green streaks or dots along the lateral veins around midribs can be observed from the infected plants. The leaves become rigid and more erect than those of healthy plants, exhibit yellowing and necrosis along their edges and show specific symptom called rosette. Suckers that emerge from infected stools are systemically infected and exhibit stunted growth, no bunches are produced.

The virus is transmitted by banana aphid, *Pentalonia nigronervosa* (Muharam 1985). Incubation period in inoculated suckers of Pisang Ambon

Hijau varies between 23-41 days after inoculation. Maryam *et al*. (1992) reported that under greenhouse conditions, the virus can be transmitted by a single aphid and 20 aphids are needed for maximum transmission. Three hours acquisition feeding period resulted to 90 % transmission. The aphid remains infective for 13 days after feeding on infected material. The life cycle is 16.4 days and the longevity is 22.6 days. Mature aphids produce average an of 5.8 nymphs per day. One of the predators found in the field is *Ancylopterix* sp.

Pisang Ambon Jepang (Giant Cavendish) and Pisang Ambon Putih (Gros Michel) are very susceptible to the virus (Muharam 1984). Recently Sulyo *et al.* (1992) reported four banana cultivars showed resistance to BBTV out of 30 cultivars tested. The cultivars were: Klutuk (BBw), Jimbluk (?), Kapas (AAB), and Seribu (AAB).

Cucumber Mosaic Virus (CMV). CMV was first observed to occur in West Java in 1978 (Sulyo *et al.* 1978, Duriat and Gantika 1979). Recently, it was found mostly in cultivars belonging to the AAB genomic group grown in Central Java, Lampung and West Sumatera. Infection in plantings is localized and seldom result to serious outbreaks. Infected plants showed chlorotic streaking symptoms. It is likely to occur where bananas are grown and not surprising therefore if the incidence varies from common to rare and the disease is often unnoticed as the symptoms are not pronounced and tend to disappear and reappear periodically. Mild attacks may lead to full recovery.

CMV is caused by a cucumovirus, 3 ssRNA + 1 subgenomic RNA and considered the second serious viral disease affecting banana and plantain in Indonesia. Visual symptoms of CMV is characterized by a conspicuous inter-veinal chlorosis of the leaves. Study on mechanical transmission through indicator plants resulted to mosaic symptom on *Cucurbita pepo* and *Datura stramonium*, necrotic local lesion on *Chenopodium amaranticolor* and *Chenopodium quinoa* (Sulyo *et al*. 1994). Common observation of infected plants is stunted growth and low yield. In severe cases this is accompanied by rotting of the heart leaf and central cylinder.

Banana mosaic is cosmopolitan and has been called various names. The incidence of cucumber mosaic infectious chlorosis, heart rot, and virus sheath rot and it is now accepted as a complex of apparently, closely related diseases.

Banana Streak Virus (BSV). Viral leaf streak of banana in Indonesia was first observed on Cavendish cultivars in West Java, and the causal agent was identified as BSV (Sulyo *et al.* 1994). Since then, BSV was reported to occur and infect non-commercial 'Pulo' cultivar (AAB) in West Sumatera and Cavendish cultivar in the province of Lampung. It appears likely that BSV is a minor problem being found only in a few plants of certain cultivars with no evidence of spread.

BSV is a member of the plant virus genus Badnavirus, representing viruses which have bacilliform particles averaging 30 x 150 nm in size and containing a circular double-stranded DNA genome 7.2 kb in size. BSV also infect sugarcane, cacao, pineapple, black pepper, and citrus (Lockhart and Olszewski 1994). The visual symptoms consist of broken or continuous streaks which vary in color from light yellow to dark brown. Symptoms induced by BSV may sometimes resemble those caused by cucumber mosaic virus.

The spread of BSV is through vegetative propagation, mealy bugs, and infected seeds. Infected plants can adversely affect yield and fruit quality. Isolates of the virus differ in symptoms produced, and are serologically and genomically

heterogenous, which has led to problems in developing reliable indexing methods for identification (Lockhart 1995).

Citrus

Table 1 indicates that five viral diseases of citrus were confirmed prevalent in Indonesia. CVPD and tristeza are recognized as the most serious threats based on current economic impact, geographical distribution, and effect on commercial cultivars (Table 2). Level of incidence and severity varied between varieties and regions depending on agro-ecological zones.

Citrus Vein Phloem Degeneration (Greening-like Disease). Among citrus disease complex, the greening disease locally known as Citrus Vein Phloem Degeneration (CVPD) is by far the most important disease of citrus in Indonesia. CVPD was recorded as an infectious disease in the 1950s which by that time was already widely distributed. Since then it has become more severe as a limiting constraint to citrus production in many areas of Indonesia. This disease is believed to have originated from India or China and has spread to the rest of Asia and Africa with introductions of cultivated citrus. The importance of CVPD was felt as early as 1948 in Pasarminggu, a center of introduction from which grafted trees were distributed in different parts of Indonesia. Survey conducted in 1984 seemed to indicate that CVPD has spread all over Java and Sumatera islands. Both the disease and the vector appeared also recently in Bali, the South Sulawesi and probably West Kalimantan and Irian Jaya. However, some of the scattered plantings in the rugged terrain of NTT are still free from infection.

CVPD is a graft and vector transmissible disease, and caused by a sivetube-restricted bacterium that could never be obtained in artificial culture. The etiology of the disease was characterized as the alpha subdivision of the Protobacteria under genus *Liberobacter* (Jagoueix *et al*. 1996). Two species of *Liberobacter* were reported namely *Liberobacter africanum* and *Liberobacter asiaticum* in Asia and South East Asia. CVPD is inducing a wide range of systemic reactions, from relatively mild to extremely severe. The major symptoms consist of leaf yellowing, leaf mottling or blotching along the leaf margin, and fruits with abortus seeds.

Citrus Tristeza Virus (CTV). Citrus tristeza is undoubtedly the most economically important virus of citrus in Indonesia. It is endemic in all citrus growing provinces, and their presence had been confirmed serologically using antibodies from either AGDIA developed by Dr. Garnier and Dr. Bove from IRFA-France. It is an aphid-transmitted virus, with long, flexuous threadlike particles 2,000 nm in length and 11 nm in diameter. It is known to exist as a complex of different isolates in the field (Lee *et al.* 1994), and serologically different strain can be detected in a CTV source (Muharam and Dwiastuti 1991). For example, a mild CTV M27 reacted strongly to two different monoclonal and polyclonal antibodies, while the same isolates from aphid-transmitted subcultures reacted with polyclonal only but not with either monoclonal antibodies (Kano 1996), which indicates that at least two different serotypes can infect single citrus plant, and that separation of certain serotypes can occur through aphid transmission.

Mild strain cross protection has been developed successfully in several countries to reduces losses from CTV stem pitting, but has not been successfully implemented in Indonesia. Several isolates of mild strains based on visual symptom have been collected. Experimentally, two CTV mild strains exhibit mild symptoms on some varieties but has failed in others. Difficulties in finding isolates which are both mild and protective and then shows no harm to other varieties / cultivars has limited practical application of mild strain cross protection. Lack of ability to predict potential hazards has also precluded preventive use of cross protection.

Recent studies on CTV indicate that more successful application of mild strain cross protection will be more complex than originally believed. At the same time, as knowledge of CTV increases at the molecular level, the option to engineer isolates with desirable properties and to eliminate liabilities, such as vector transmissibility is rapidly increasing. Development of design protecting isolates could provide creative solutions for a number of CTV problems. Cross protection program for the CTV preimmunized in Indonesia has been reoriented. Huge exploration of CTV isolates has been carried out to find biological diversities of the virus. Three groups of severe, moderate, and mild strain of CTV that produces stem-pitting, vein clearing and seedling yellow respectively have been collected. At the same time, purified CTV isolates maybe isolated through serial host passage with aphid transmission.

Citrus Exocortis Viroid (CEV). Exocortis, a minor viral disease of citrus in Indonesia is present in almost all citrus growing centers. Although many commercial cultivars are symptomless carriers, if the viroid is present in trees, stunted growth can be observed to various degrees on most rootstocks. CEV is transmitted mechanically by cutting tools from citrus to citrus. Dwiastuti demonstrated that a low dilution of sodium hypochlorite is sufficient to disinfect cutting tools contaminated with CEV.

Citrus Psorosis Virus (CPV). Psorosis was previously considered to be caused by a complex of viruses including psorosis, a bark scaling, concave gum, blind pocket, crinkly leaf, and infectious variegation. However, recent evidence suggests they are all separate diseases. Infected trees showing bark scaling symptoms suspected to be infected by the virus were found in some orchards in Bali, and Central and East Java.

Citrus Vein Enation-Woody Gall (CVEV). In Indonesia, woody gall symptoms were found in many citrus orchards in Java. Because of being aphids transmissible like CTV, it is suspected that the virus is widely distributed in citrus growing areas. The virus induces vein enation on leaves of Mexivan lime and Sour orange and galls which commonly occur on Rough lemon. Vein enation and woody gall diseases of citrus are known to be induced by the same virus.

Indexing Techniques

As it is well known, indexing program is an important component for the production of disease-free planting materials. Development of reliable and rapid diagnostic tests for important viral and bacterial pathogens to prevent their unintentional geographic distribution to the wider areas is therefore needed. Current implementation of indexing techniques in Indonesia is summarized in Table 3.

Table 2. Prevalence of banana and citrus virus disease in each province.

Bacteria/Virus/ Virus-Like	Natural Spread	Vector	*Geographical Distribution	**Current Impact	***Potential Impact
BBTV	Yes	Aphids	Wide	Moderate	Large
CMV	Yes	Aphids	Wide	Small	Small
BSV	Yes	Mealybug	Localized	Small	Small
CVPD	Yes	Psylla	Wide	Large	Large
CTV - seedling yellow - stem pitting - vein clearing	 Yes Yes Yes	 Aphids Aphids Aphids	 Localized Moderate Wide	 Moderate Moderate Moderate	 Moderate Moderate Moderate
CEV	?		Localized	Small	Small
CPV	?		Localized	Small	Small
CVEV	Yes	Aphids	Localized	Small	Small

* Wide: found in more than 50 % of the growing regions in the province, localized: found in less than 50 % of the growing regions in the province
** Current economic impact based on current geographical distribution and effect on commercial cultivars
*** Estimate of future economic effects assuming disease and vector become widespread

BBTV : Banana Bunchy Top Virus
CMV : Cucumber Mosaic Virus
BSV : Banana Streak Virus
CVPD : Citrus Vein Phloem Degeneration
CTV : Citrus Tristeza Virus
CPV : Citrus Psorosis Virus
CEV : Citrus Exocortis Viroid
CVEV : Citrus Enation - Woody Gall

Banana

Virus identification on banana is mostly done on symptomatology basis. However, some private nurseries are now implementing serological technique by using antibodies which are commercially available from AGDIA. The antibody is suitable for use in ELISA and in general, has a broad spectrum of reactivity with BBTV and CMV.

However, it was observed from evidences that isolates showing mild and symptomless strains of BBTV are detectable with some antibodies, but not all. This indication pushed us to the reorientation of indexing program for BBTV and CMV. Much research work has been undertaken on the studies of biological diversities of virus strains and development of indexing techniques recently. Significant progress has been obtained by producing BBTV and CMV antibodies which have significant reactivity with some isolates. Although further studies still have to be conducted, additional techniques will be adopted as they become available.

Citrus

Unlike banana, indexing techniques for citrus virus diseases have been implemented under the ICVIP program since 1987 (Muharam and Whittle 1989). It has since then improved and new techniques are now available. Indexing was particularly developed for CVPD and tristeza, since both diseases have an important economic impact and widespread in Indonesia. We have routinely been using DAS ELISA followed by the general protocol of Clark and Adam (1977) for CTV, by using commercial antibodies available (AGDIA). PCR procedure follows the protocol of Bove and Garnier (1996) developed for CVPD disease.

Table 3. Current implementation of indexing techniques for banana and citrus viral disease in Indonesia

Bacteria/Virus Virus-like	Indexing methods		
	Indicator Plant	ELISA (protocol)	PCR (protocol)
BBTV	-	Clark and Adam (1977)	-
CMV	-	Clark and Adam (1977)	-
BSV	-	-	-
CVPD	-	-	Bove and Garnier (1996)
CTV: - Seedling Yellow - Stem Pitting - Vein Clearing	Eureka lemon Sweet orange, grape fruit Mexican Lime	Clark and Adam (1977)	-
CEV	Etrog Citron	-	-
CPV	Madame Vinous	-	-
CVEV	Mexican lime	-	-
Xyloporosis	Parson Special Mandarin	-	-

BBTV : Banana Bunchy Top Virus
CMV : Cucumber Mosaic Virus
BSV : Banana Streak Virus
CVPD : Citrus Vein Phloem Degeneration
CTV : Citrus Tristeza Virus
CEV : Citrus Exocortis Viroid
CPV : Citrus Psorosis Virus
CVEV : Citrus Enation - Woody Gall

Integrated Management of Banana and Citrus Viral Diseases in Indonesia

Banana

Since the occurrence of BBTV and CMV affecting major commercial cultivars in Indonesia, campaign on viral disease management has been intensified during the last five years. Virus-free propagating materials are prerequisites for the establishment of new banana plantings. For established plantings, effective control of the diseases requires early detection and immediate eradication of infected plants followed by replanting with disease-free planting materials. As most banana virus produces character symptoms on the leaves, eradication was commonly done under symptomatology basis.

An increasing huge demand for the tissue-culture propagated plant materials recently stimulated the development of unregistered *in vitro* private nurseries. Problems of quality control arises as production of planting materials is done in an economical rather than technical considerations. The only private nursery applying indexing procedures is in Bogor. A tissue culture propagation system incorporated with a virus indexing technique is then acting as a particular means of viral disease control in banana.

In 1990, collaborative research with Dr. Lockhart from the University of Minnesota has allowed the development of BSV indexing technique (Sulyo *et al*. 1994). This was intensified, following assistance visits of CIRAD-FLHOR, Montpellier (Tezenas du Montcel *et al*. 1996) to set up research priority for banana viral diseases in Indonesia. Virus indexing procedures for banana viruses, classical micropropagation through tissue culture including Temporary Immersion System as a new way for massive propagation are among research activities that are now being initiated (Figure 1).

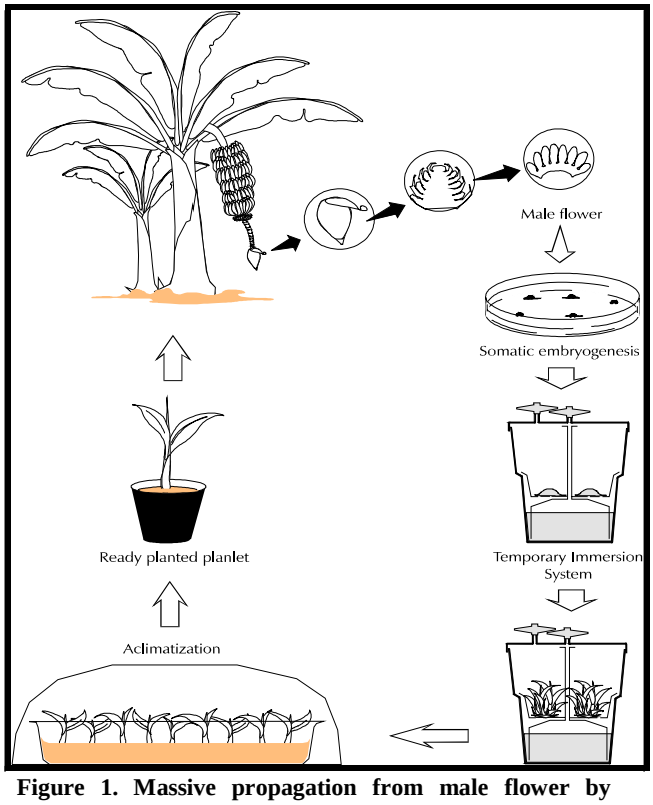


Figure 1. Massive propagation from male flower by temporary immersion system.

Citrus

Efforts to control viral diseases have been initiated since 1970, but were curtailed before any positive results were obtained. Until 1985, when UNDP/FAO regional citrus greening network among Southeast Asian countries was established, research on the disease control allowed on the understanding of the intricate interrelationships of the factors influencing disease epidemic (Gottwald and Aubert, 1987). Thirteen years of experiences of controlling citrus systemic disease in Southeast Asia allowed better understanding of disease epidemic. Quantitative analysis of epidemic components has led to the formulation of disease control strategy. Implementation of the program in Indonesia has started since 1987 when the first huge program namely Indonesia Citrus Variety Improvement Program (ICVIP) was established. The program consists of three main strategies i.e. production of disease-free planting material; eradication of infected plant and replanting with disease-free planting materials; and effective control of insect vector.

Production of disease-free planting material

The life-span of citrus trees in Indonesia is short due to systemic diseases and cannot be cured once the plants became infected. Therefore, the production of disease-free planting material is of critical importance in citrus rehabilitation program. Production and dissemination system of disease-free planting materials is presented in Figure 1.

The shoot tip grafting (STG) technique developed by Navarro *et al.* (1975) and modified by Dr. Hong Ji Su has been adopted for cleaning-up desirable citrus varieties. Successful grafts are re-grafted one month later onto young and vigorous seedlings in a warm screenhouse to promote growth and flushing. The selection of mother trees is based primarily on horticultural qualities of each variety rather than on initial phytosanitary standards. The variety collection currently contains 67 varieties of mandarin, 35 of sweet orange, 8 pummelos, 22 of grapefruit and others.

Although the shoot tip size is 0.1 to 0.2 mm, most of STG plants i.e.: 19% of mandarins and 45.1% of sweet oranges are still infected by CTV. These diseases are not always eliminated during the production of *in vitro* plants using shoot tip culture, leading to disastrous consequences if mother plants are not virus free. Therefore, indexing programs is a crucial step and particular prerequisite for producing disease-free plant materials. As an important component of the ICVIP regarding the establishment of virus-free budwoods, indexing programs have focused on: 1). Indexing of shoot tip source plants for non vectors transmissible pathogens, i.e.,: exocortis, xyloporosis, and psorosis, 2). Indexing of re-grafted plants originating from STG for vector transmissible pathogens i.e.: greening (CVPD), CTV and vein enation / woody-gall, and 3). Indexing for all viruses of mother plants in Foundation Blocks.

Foundation Blocks (FB) as the primary sources of bud wood consisting of trees grafted with virus-free material. Under the program, four blocks are envisioned in strategic areas and implemented in size and composition to meet the expected demands. These FBs are being established in Zone 1 in East Java, in Zone 2 in Riau, in Zone 3 in West Kalimantan and Zone 4 in South Sulawesi (Figure 2). A subsidiary screenhouse-protected block has been established in Bali to serve the special requirements of that province for immediate replanting. All FBs are government-owned and isolated to reduce reinfection risks, and subject to regular indexing.

Given the high costs of FB maintenance, and very large number of bud required, a system of bud wood multiplication is essential. A bud-wood Multiplication Block (BMB) consists of densely planted small trees produced FB from buds serves as direct source of buds for nurseries. To reduce reinfection risks, such blocks can only be harvested over a three-year period before eradication. Although in principle BMBs can be privately owned, the authorities in Bali and East Java, West Java and West Sumatera, have kept them under government ownership. In the province of Riau, however, BMBs are being maintained by private nurseries.

The system for the distribution of virus-free bud wood and stocks from FBs to the growers is inefficient such that some risks of reinfection cannot be avoided. To ensure that the plants available to growers retain their phytosanitary quality, a vector-disease monitoring and certification program is being implemented by related Institutes. Inspection and

certification are regularly done by concerned institutions. based on intensive inspection at critical periods. However, a crucial aspect has been the development of nurserymen's associations through which information can be channeled to increase the professionalism within the sector.

The implementation of the program resulted into some improvement at one region in West Java as shown by the increasing productivity of about 200 % as compared with national productivity. However at one region in Bali Province the achievement was not fully successful.

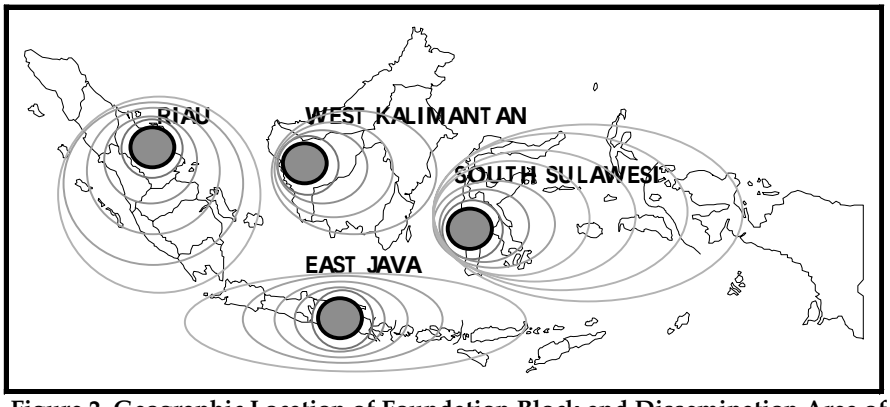


Figure 2. Geographic Location of Foundation Block and Dissemination Area of Bud-wood

Eradication of infected plant and replanting with disease-free planting materials

The control of CVPD disease depend largely on the control of vector and eradication programs. Eradication is one of several alternatives or complementary means of control. Eradication procedures are done as part of a carefully integrated control program involving a sequence of measures, each of which may be of limited value when adopted alone. From our 15 years of experience in controlling CVPD, the effective measures of eradication is through the introduction of eradication measures at an early stage in the development of an epidemic and implementation of an integrated series of control measures. An IPM approach for a long period of time could reduce the spread of the diseases.

Most citrus are grown in smallholdings. The replanting of an individual orchard usually surrounded by existing infected orchards are bound to fail. With CVPD being at epidemic levels, resulting in a high inoculum and almost uncontrolled vector populations, sanitation is of little value, because of the delay between infection and symptom expression. Reducing the inoculum by eradicating infected trees and replanting large areas is necessary. Replanting has to be done immediately to reduce the period of mixed plantings. The strategy to release virus-free planting must coincide with an eradication program.

Vector Control

With the assumption that disease-free planting material is available through STG the next immediate problem is what to do with existing infected orchards. The experience in Indonesia, especially among small farmers, is to leave infected trees in the orchard until they finally cease production. Such trees, while consuming production inputs without giving a return, act as source of inoculum for the spread of the disease.

The success of a citrus rehabilitation program depends mostly on the successful control of vectors and the reduction of inoculum. The aim of controlling vectors is to reduce the rate of disease spread. The initial disease progress rate is related to nurserymen and farmers' initiatives to remove the source of inoculum while the epidemic pattern is linked to the natural transmission by the vector. As single vector is capable to transmit CVPD-causal-bacterium, the tolerance population of *D. citri* must be at zero level. This means that being a vector of CVPD, it should never be present in a citrus orchard. However, this idea is unattainable in practice, so that strategy of vector control is to hold the vector populations as low as possible.

D. citri does not cause significant mechanical damage or characteristic symptoms on the leaves, therefore, citrus farmers or even extension officers usually overlooked its presence in the orchard. Farmers are usually more ready to spray non target pests rather than *D. citri* that is difficult to recognize. As a result, spraying, if done at all, is done in an irrational way and is usually aimed at non target pests with a resulting speculative effect on vector populations. The behavioral response of citrus psylla has allowed the development of a method for predicting adult psylla population. Fluorescent yellow sticky traps and flushing rhythmically can serve as a warning system showing whether citrus psylla has an outbreak potential so that control decisions can be made.

Another important factor in regulating population build up of psylla is natural enemies. Undoubtedly, *Tamarixia radiata, Diaphorencyrtus aligharensis, Hirsutella* sp., *Beuveria* sp., *Metharrizium* sp., and several species of predators are among the potential natural enemies which contribute significant impact in regulating vectors. Their valuable contribution can be shown from their ability to suppress population of *D. citri* at some places in East Java, West Kalimantan, West Sumatera, and East Nusa Tenggara. However, from the disease control point of view, it appears unlikely that citrus psylla can be controlled only in a natural way. Therefore, it is an important strategy to hold its populations as low as possible especially during critical period (June) where the proportion of viruliferous vectors is high (Bove *et al.* 1996).

Various insecticides with certain application technique are effective against vectors and reduce the adverse effect on beneficial insects. Dimethoate applied as soil drenches and monocrotophos as a bark paint will provide protection for up to four weeks and a good component of integrated control program. The use of dimethoate as a soil drenching for more than 0.05% active ingredient will cause damage so that comprehensive trials are needed before it is used. Integrating the role of parasites in vector control, developing a monitoring program, and implementing pesticide resistance management through IPM practice will form part of future research activities.

Conclusion

Banana and citrus are important commercial fruit crops in Indonesia, the priorities in agribusiness development are set. The market demand for both commodities in the domestic and international scene continues to increase during the last five years. Productivity of either banana or citrus is low compared to banana- and citrus-producing countries in Southeast Asia. Five viral diseases including bacteria, viroid and virus like diseases on citrus, and three virus diseases on bananas have been confirmed in Indonesia. Among them, CVPD and tristeza on citrus, and BBTV and CMV on bananas are recognized as the most serious threats to their respective crops causing substantial losses. Control strategy aimed to eradicate these diseases has established three particular components: production of disease-free planting material, effective and efficient control of the vector, and complementary sanitation campaign. While these are being carried out separately, any one of these three tactical actions might result in some temporary improvement, but for a durable and sustainable results, they must be integrated.

References

Aubert, B. Epidemiological aspects of the greening (Huanglungbin) disease in Asia. Workshop on Citrus Greening Huanglungbin Disease. Fuzhou-China, Dec. 6-12. 1987 (Review and Abstract).

Becu, P.; Whittle, dan A.M. Research and Development for Citrus Rehabilitation. The Indonesian Citrus Variety Improvement Programme-Costing Study. INS / 84 / 007-Field Document 1. AARD-FAO, 1988. 77p.

Bove, J.M.; Garnier, M. Rapid Detection of *Liberobacter asiaticum*, the Causal Agent of Citrus Huanglongbing Disease (Greening, Citrus Vein Phloem Degeneration), in Bali, Indonesia. Report of Mission, North Bali Ground Water Irrigation Water Supply Project, 1996. 24.p.

Clark, M.F.; Adams, A.N. Characteristic of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483, 1977.

Dietzen, R.G. and Thomas, J.E. Properties of virus-like particles associated with banana bunchy top disease in Hawaii, Indonesia and Tonga. Australian Plant Pathology. 20:161-165, 1991.

Dwiastuti, M.E. Pengaruh Lama Kontaminasi Viroid Exocortis Jeruk pada Pisau terhadap Keberhasilan Penularan dan Uji Inaktivasi CEV secara Kimiawi. Kongres Nasional XIII dan Seminar Ilmiah PFI. Mataram 27-29 September 1995. (ed. Parman, A. Rohyadi, I.G.E. Gunartha, Murdan, L. Irasakti, M. Sarjan dan W. Wangiyana), 1995. p. 398-403.

Frison, E.A.; Putter, C.A.J.(Eds). FAO / IBPGR technical guidelines for the save movement of Musa germplasm. FAO-UN, Rome / IBPGR,Rome, 1989. 23 p.

Gottwald, T.R.; Aubert, B.; Mercado, B.G. Prelimenary assessment of the spatial distribution of citrus greening in plots in Shantou, China, and Luzon, Philippines. In: Proc. 2nd FAO-UNDP Regional Workshop Lipa Philippines Asian / Pacific Citrus Greening. 20 th - 26th. 1988

(ed. Ke Chung and Aubert).

Jones, D.R.; Lockhart, B.E.L. Banana Streak Disease. *Musa* Disease Fact Sheet No. 1. INIBAP, 1993. 1 p.

Jagoueix, S.; Bove, J.M. ; Garnier, M. PCR detection of the two 'Candidatus' liberobacter species associated with greening disease of citrus. Molecular and Cellular Probes 10:43-50, 1996.

Lockhart, B.E.L.; Olszewski, N.E. Badnavirus Group. Pp. 139-143 in Webster, R.G. and A. Granoff (eds.). Ecyclopedia of Virology, Vol I. Academic Press, New York, 1994. 516 pp.

Lockhart, B.E.L. Banana streak Badnavirus Infection in *Musa*: Epidemiology, Diagnosis and control. International Symposium on 'Integrated Management of Insect-borne Virus Diseases in Tropical Fruits. Dec. 11-16, 1995. 12 pp.

Badnavirus Group. pp. 139-143 in Webster, R.G. and A. Granoff (eds.). encyclopedia of Virology, Vol I. Academic Press, New York. 516 pp.

Muharam, A.; Whittle, A.M. Indexing of Citrus for Major Systemic Pathogens in Indonesian Citrus Variety Improvement Programme. In: Proc. Asian Citrus Rehabilitation Conference. 4th -14th July. 1989. (ed. Setyobudi L. And A.M. Whittle).

Muharam, A. Test of Resistance of some Banana Cultivars to Banana Bunchy-top Disease. Bulletin Penel. Hort. XI (4):17-20, 1984.

Muharam, A. Effects of Number of Banana Aphid (*Pentalonia nigronervosa*) nymphs on the Causal Agent Transmission of Banana Bunchy-top Disease. Bulletin Penel. Hort. XII(1):25-28, 1985.

Sulyo, Y.; Muharam; A., Rahardjo; Lockhart, B.E.L. Laporan Pertama mengenai Kehadiran Banana Streak Virus (BSV) pada Tanaman Pisang di Indonesia. Prosiding Simposium Hortikultura Nasional. (ed. T. Wardiyati, Kuswanto, S. Notodimedjo, L. Soetopo dan L. Setyobudi) PHI-Faperta Unibraw. P:550-553, 1994.

Tezenas du Montcel; Mourichon, H.X.; Escalant, J.V. Bananas and other Fruits Research and Development Acitivities in Indonesia. ARMP-AARD. Ministry of Agriculture, 1996. RI. 45.p.

Thomas, J.E.; Iskra-Caruana, M.L.; Jones, D.R. Banana Bunchy Top Disease. Musa Disease Fact Sheet No.2. INIBAP, 1994. 1 p.

Workshop Recommendations

Workshop Recommendations

Banana

Research

- Improved propagation techniques for efficient plantlet production
- Strain diversity of major viruses (BBTV, CMV, BBrMV, BSV)
- Cross protection studies
- Susceptibility of Tissue Cultured plantlets to disease infection in the field
- Yield loss assessment studies among important viruses
- Development of virus-resistant varieties

Development

- Establishment of National Seedling Cerfication Program
  - establishment of Virus Indexing Centers
  - accreditation of Banana Tissue Culture Laboratories
  - establishment of foundation stocks in insect-proof screenhouses
- National banana virus diseases eradication program
- Quarantine regulation and enforcement

Policy Recommendations

- Accreditation of Tissue Culture laboratories
- Establishment and accreditation of virus indexing laboratories
- Quarantine regulation on movement of planting materials

Others

- Publication of manuals
- Involvement of Non-Government Organizations (NGOs) in propagation and sale of virus-free materials
- Training on advanced technologies in banana virology

Citrus

Development

- Regional standardized manual of production system for disease-free planting materials and certification for citrus
  - Nursery Manual
    - Foundation block
    - Multiplication block
    - Management of healthy nursery
  - Field Manual
- Standardize Indexing Technique by establishing a centralized regional laboratory for each country
- Training - indexing, grafting, nursery management, handling database characterization of genetic materials
- Regular meetings, workshop, and creation of Asia & Pacific Technical Working Group (TWG) for Citrus

Annexes

Regional Workshop on Disease Management of Banana and Citrus: The Use and Management of Disease-free Planting Materials

Program

Day 1: October 14
Morning Session

8:30 – 10:00	Registration
10:00 – 12:00	Opening Ceremonies Master of Ceremonies: *Mr. Angelito T. Carpio* Science Research Specialist, PCARRD
	Welcome Address *Dir. Nerius I. Roperos* Director, Bureau of Plant Industry, Philippines
	Opening Address *Dr. Iwao Watanabe* Deputy Director, FFTC, Taiwan
	Keynote Address *Dr. William D. Dar* Acting Secretary, Department of Agriculture, Philippines
	Workshop Rationale *Dr. Agustin B. Molina* Regional Coordinator INIBAP-ASPNET
	Group Photograph
12:00 - 1:00	Lunch Break
Session I	**Chairperson:** ***Dr. Chan Hock Teo***
1:00 - 1:30	Epidemiological Review on Citrus Greening and Viral Diseases of Citrus and Banana with Special Reference to Disease-free Nursery System *Dr. Hong-Ji Su* National Taiwan University, Taiwan
1:30 - 2:00	Production and Cultivation of Virus-free Banana Tissue-cultured Plantlets in Taiwan *Dr. Shin-Chuan Hwang* Taiwan Banana Research Institute, Taiwan

2:00 - 2:30 Viruses of Banana and Methods for their Detection
Dr. John Thomas
Queensland Department of Primary Industries, Australia

2:30 - 2:50 Virus and Virus-like Diseases of Banana and Citrus in Malaysia: Status and Control Strategies
Dr. Ching-Ang Ong
Malaysian Agricultural Research and Development Institute, Malaysia

2:50 - 3:20 Open Forum

3:20 - 3:50 Coffee / Tea Break

Session II **Chairperson:** ***Dr. Woo Nang Chang***

3:50 - 4:10 Disease Management of Citrus Orchards Planted with Disease-free Seedlings in Thailand
Dr. Suchat Vichitrananda
Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand

4:10 - 4:30 Recent Progress in the Research on Citrus Greening in Asia Including a Serological Diagnosis
Dr. Yoshihiro Ohtsu
National Institute of Fruit Tree Science, Japan

4:30 - 5:00 Ecology of the Insect Vectors of Citrus Systemic Diseases and Their Control in Taiwan
Dr. Chiou-Nan Chen
National Taiwan University, Taiwan

5:00 - 5:20 Citrus Greening Control Project in Okinawa, Japan
Dr. Shinji Kawano
Okinawa Perf. Agricultural Experimental Station, Japan

5:20 - 6:00 Open Forum

6:30 Welcome Dinner hosted by FFTC

Day 2: October 15
Morning Session

Session III **Chairperson:** ***Dr. John Thomas***

8:00 - 8:30 Management of Viral Streak in Banana and Plantain: Understanding a New Challenge
Dr. Ben Lockhart
University of Minnesota, USA

8:30 - 8:50 Pathological and Molecular Characterization of BBTV Strains in Asia
Dr. Hong-Ji Su
National Taiwan University, Taiwan

8:50 - 9:10 The Impact of Tissue-cultured Plants in the Ongoing Eradication and Rehabilitation Program in the Philippines
Ms. Lydia Magnaye
Bureau of Plant Industry, Davao, Philippines

9:10 - 9:30 Epidemiology and Integrated Management of Abaca Bunchy Top in the Philippines
Dr. Avelino Raymundo
University of the Philippines Los Baños, Philippines

9:30 - 9:50 Rehabilitation of BBTV-affected Areas in the Philippines: Experiences and Problems
Dr. Rene Rafael C. Espino
University of the Philippines Los Baños, Philippines

9:50 - 10:10 Open Forum

10:10 - 10:30 Coffee / Tea Break

Session IV **Chairperson:** ***Dr. Marina P. Natural***

10:30 - 10:50 Status of Disease Management of Citrus in the Philippines
Mr. Ceferino Baniqued
Philippine Phytopathological Society Inc., Philippines

10:50 - 11:10 Management of Citrus Disease-free Seedings in Southern Vietnam
Ms. Le Thi Thu Hong
Southern Fruit Research Institute, Vietnam

11:10 11:30 Management of Citrus Disease-free Seedlings in Northern Vietnam
Dr. Ha Minh Trung
National Institute of Plant Protection, Vietnam

11:30 11:50 Establishment of Disease-free Foundation and

Nursery for Controlling Greening Disease and Citrus Tristeza Virus: A Sarawak Experience
Mr. Chan Hock Teo
Agriculture Research Center, Sarawak, Malaysia

11:50 12:10 Status of Banana and Citrus Viral Diseases in Indonesia
Mr. A. Nurhadi
Research Institute for Fruits, Indonesia

12:10 12:30 Open Forum

12:30 1:30 Lunch Break

Afternoon Session

1:30 4:30 Workshop Sessions
Banana Group Facilitators:
Dr. Ramon V. Valmayor and
Dr. Shin-Chuan Hwang

Citrus Group Facilitators:
Dr. Hong Ji Su and
Dr. Florendo C. Quebral

4:30 5:30 Presentation of Output (Synthesis)

5:30 6:00 Closing Session

Concluding Remarks
by: *Dr. Iwao Watanabe*
Deputy Director, FFTC

Concluding Remarks
by: *Dr. Agustin B. Molina*
Regional Coordinator, INIBAP-ASPNET

Distribution of Plaques and Certificates
Master of Ceremonies: *Mr. Angelito T. Carpio*

6:30 Farewell Dinner hosted by PCARRD

Day 3: October 16
Field Trip

Day 4: October 17
Departure of Participants

List of Participants

Special Guest

Dr. William D. Dar
Acting Secretary
Department of Agriculture
Diliman, Quezon City, Philippines

Mr. Nerius I. Roperos
Director, Bureau of Plant Industry
Department of Agriculture
692 San Andres
Malate Manila, Philippines
Tel. No. (63)(02) 525-7857
Fax No: (63)(02) 521-7650
E-mail: bpinir@info.com.ph

Speakers

Australia

Dr. John E. Thomas
Principal Plant Virologist
Queensland Horticulture Institute
Department of Primary Industries
80 Meiers Road, Indooroopilly
Queensland, 4068 Australia
Tel. No.: 61-7-3896-9371
Fax No: 61-7-3896-9533
E-mail: thomasje@dpi.qld.gov.au

Indonesia

Dr. Nurhadi
Plant Protection Coordinator
Research Institute for Fruits
Agency for Agricultural Research and Development
JI Raya Solok-Aripan Km
Solok 27301
West Sumatra, Indonesia
Tel. No: 63-0755-21037
Fax No: 63-0755-20592
E-mail: rif@padang.wasantara

Japan

Dr. Shinji Kawano
Researcher, Okinawa Prefecture Agriculture
Experimental Station – Miyako Branch
2071-40 Nishizalo, Hirara 906-0012m Okinawa, Japan
Tel. No: 81-9807-2-2033
Fax No: 81-9807-2-8064
E-mail: kawan@nousi.prcf.okinawa.jp

Dr. Yoshihiro Ohtsu
Chief, Laboratory of Plant Pathology
National Institute of Fruit Tree Science
Fujimoto 2-1, Tsukuba
Ibaraki 305-8605 Japan
Tel. No: 81-298-38-6546
Fax No: 81-298-38-6541
E-mail: yotsu@fruit.affrc.go.jp

Malaysia

Dr. Ching-Ang Ong
Senior Research Officer
Malaysia Agricultural Research and Development Institute
P.O. Box 12301 GPO, 50774
Kuala Lumpur, Malaysia
Tel. No. : 943-7428
E-mail: caong@mardi.my

Mr. Chan Hock Teo
Senior Research Officer
Agriculture Department, Agricultural Research Centre
Semongok, PO Box 977, Kuching
Sarawak, Malaysia 93720
Tel. No.: 082-611-1711
Fax No: 082-611-178

Taiwan

Dr. Chio-Nan Chen
Professor
Department of Entomology, National Taiwan University
27 Lane 113 Roosevelt Rd. Sec. 4
Taipei 106 Taiwan
Tel. No: 886-2-2732-3677
Fax No: 886-2-2732-3677
E-mail: coachen@ccms.ntu.edu.tw

Dr. Shin-Chuan Hwang
Director, Taiwan banana Research Institute
Chiuju, Pingtung, Taiwan 90403
Tel. No: (08) 739-2113
Fax No: (08) 739-3647
E-mail: tbri@ksts.seed.net.tw

Prof. Hong-Ji Su
Professor
Department of Plant Pathology
National Taiwan University
Taipei, Taiwan
Tel. No: 886-2-236-5717
E-mail: hjsu@ccms.ntu.cdu.tw

Thailand

Mr. Suchat Vichitrananda
Plant Pathology and Microbiology Division
Department of Agriculture
Chatuchak, Bangkok 10900 Thailand
Tel. No: (66-2) 579-9582
Fax No: (66-2) 940-6371

USA

Dr. Benham E. Lockhart
Professor
Department of Plant Pathology
University of Minnesota
495 Borlaug Hal, St. Paul
MN 55108 USA
Tel. No: 1- 612-625-5785
Fax No: 1- 612-625-9728
E-mail: plpa@puccini.crl.unn.edu

Vietnam

Ms. Thu Hong T. Le
Head, Research Management and International Cooperation
Southern Fruit Research Institute
Chau Thans-Tiengiang, Vietnam
Tel. No 84—7385-5158
Fax No: 84-73-855588
E-mail: SOFRI@Nctnamz.org.vn

Mr. Minh Trung Ha
Deputy Director for Research
National Institute of Plant Protection
Chem Tu Liem, Hanoi, Vietnam
Tel. No: 84-4834-4724
Fax No: 84-4836-3563
E-mail: nipp.vnd@bdcn.net

Philippines

Ms. Lydia V. Magnaye
Supervising Agriculturist
Bureau of Plant Industry-
Davao National Crop Research and Development Center
Bago-Oshiro, Davao City, Philippines
Tel. No: 63-82 293-0107
Fax No: 63-82 293-0107
Email: banphil@dv.weblinq.com

Dr. Avelino D. Raymundo
Associate Professor, Department of Plant Pathology
College of Agriculture, UP Los Baños
College, Laguna, Philippines
Tel. No: (63 49) 536-3534
Fax No: (63 49) 536-3534
E-mail: billyray@laguna.net

Dr. Rene Rafael C. Espino
Professor, Department of Horticulture
College of Agriculture, UP Los Baños
College, Laguna, Philippines
Tel. No. (63)(49) 536-2448
Fax No: (63) (49) 536-2478
E-mail: butchoy@laguna.net / rrce@mudspring.uplb.edu.ph

Mr. Ceferino A. Baniqued
Center Chief IV, BPI
Bureau of Plant Industry
Baguio City, Philippines
Tel. No: (63) 74 442-5270
Fax No: (63) 74 443-3540

Local Participants

Mr. Alejandro R. Muñez
Associate Scientist, STANFILCO-Dole (Phils), Inc.
Doña Socorro St., Belisario Hts. Subd.
Lanang, Davao City, Philippines
Tel. No. 233-1327 (4377)

Dr. Sai Leng
Senior Scientist, STANFILCO-Dole (Phils), Inc.
Doña Socorro St., Belisario Hts. Subd.
Lanang, Davao City, Philippines
Tel. No. (63 82) 234-0241
Fax No: (63 82) 233-1512
E-mail: sai-leng@na.dole.com

Mr. Romulo S. Palcon
Regional Director
Fiber Industry Development Authority
Gima Bldg., Pichon St.
Davao City, Philippines
Tel. No: (63 82) 227-4544 / 227-666
Fax No: (63 82) 227-4544

Mr. Jose P. Marfori
Manager, Davao Orchid Lab & Garden

Dizon Comp., Bacaca
Davao City, Philippines
Tel. No: (63 82) 227-9915
Fax No: (63 82) 226-4619
E-mail: jpm@gateway.com

Mr. Rodolfo G. Atabug
Research Manager
Tristar Plantation / Davao Trade Exponents Inc.
505 Aguho St., Juna Subd
Matina, Davao City, Philippines
Tel. No. (63 82) 299-2986 to 87
Fax No: (63 82) 299-2996

Mr. Gilbert Cumila
Project Supervisor
CE-Casecnan Water & Energy Company
Muñoz, Nueva Ecija, Philippines
Tel. No. (63 44)456-0041
Fax No: (63 44) 892-5825 / 812-6293

Dr. Maria Emilia Rita G. Febregar
Group Manager for R&D
Lapanday Foods Corp.
Maryknoll Road, Lanang
Davao City, Philippines
Tel. No. (63 82) 235-2551
Fax No: (63 82) 234-2359
E-mail: etfabreg@webling.com

Ms. Czaryl Violeta D. E. Mendoza
Asst. Manager
Lapanday Food Corp
Research Department
Mandug, Davao City or
Maryknoll Road, Lanang
Davao City, Philippines
Tel / Fax No: 0912-704-1572
E-mail: lcfrd@skynet.net

Mr. Fernando D. Sison
Manager, Malabing Valley MPCI
Malabing, Kasibu
Nueva Vizcaya, Philippines
Tel. No. 0918-214-2100

Dr. Claro Q. Torres
St. Plant Pathologist, Dole Asia Ltd.
Doña Socorro St., Belisario Hts. Subd.
Lanang, Davao City, Philippines
Tel No: (63)(82) 234-0241
Fax No: (63)(82) 233-1512

Ms. Victoria B. Palacio
Supervisor
Marsman Estate Plantation, Inc.
Mepi, Sto. Tomas
Davao Del Norte, Philippines
Tel. No: (63 84) 829-1436
Fax No. (63 84) 829-1431

Ms. Elizabeth Lipat
Tissue Culture Supervisor
Marsman Estate Plantation, Inc.
Mepi, Sto. Tomas
Davao del Norte, Philippines
Tel. No. (63 84) 829-1436
Fax No. (63 84) 829-1431

Mr. Gomersindo M. Bulaong
Operations Manager
Diamond Farms, Inc.
Malayan House, Lanang
Davao City, Philippines
Tel. No. (63 82) 226-361 / 226-4526
Fax No: (63 82) 226-3661

Mr. Warlito A. Subayno
General Manager
Diamond Farms, Inc.
Malayan House, Lanang
Davao City, Philippines
Tel. No: (63 82) 226-3661 / 226-4526
Fax No: (63 82) 226-3661

Mr. Francisco B. Ramos, Jr.
General Manager
Davao Golden Pummelo, Inc.
51 Arellano St.
Davao City, Philippines
Tel. No.: (63 82) 224-2225 / 226-2820
Fax No.: (63 82) 224-2225

Mr. Amancio D. Manceras
Asst. Professor IV
Department of Plant Breeding and Genetics
University of Southern Mindanao
Kabacan, Cotabato, Philippines

Mr. Harem R. Roca
Asst. Professor IV, Dept. of Horticulture
Univ. of Southern Mindanao
Kabacan, Cotabato, Philippines
Tel. No.: 248-2339
Mobile Phone: (63) 0912-735-6029
E-mail: usm@webling,com

Ms. Emma K. Sales
Associate Professor V
University of Southern Mindanao
Kabacan, Cotabato, Philippines

Ms. Brigida G. Mercado
Integrated Senior Agriculturist
DA-Southern Tagalog Integrated Agricultural
Research Center (DA-STIARC)
Maraouy, Lipa City, Philippines
Tel. No.: (63 43) 756-6597
Fax No.: (63 43) 756-4962

Ms. Eugenia U. Perez
Agricultural Center Chief II
DA- Southern Tagalog Integrated Agricultural
Research Center (DA-STIARC)
Maraouy, Lipa City, Philippines
Tel / No.: (63 43) 756-6597
Fax No.: (63 43) 756-4962

Dr. Teresita M. Espino
Professor / Researcher, BIOTECH
UP Los Banos, College
Laguna, Philippines
Tel. No.: (63 49) 536-1612 / 536-2721 -23
Fax No.: (63 49) 536-2721

Dr. Marina P. Natural
Chair & Associate Professor
Dept. of Plant Pathology
College of Agriculture, UP Los Banos
College, Laguna, Philippines
Tel. No.: (63 49) 536-3534
Fax No.: (63 49) 536-3534
E-mail: mnatural@laguna.net

Dr. Narceo B. Bajet
Associate Professor, Dept. of Plant Pathology
College of Agriculture, UP Los Banos
College, Laguna 4031, Philippines
Tel No.: (63 49) 536-3534
Fax No.: (63 49) 536-3534
E-mail: nbb@medspring.uplb.edu.ph

Ms. Analyn D. Bolivar
Senior Agriculturist
Fiber Industry Development Authority
Pichon St., Davao City, Philippines
Tel. No.: 227-6666

Mr. Wilfredo G. Espada
Consultant
Comval Tropical Fruit Inc.
FSDizon Farms, Inc.
Riverview, Bacaca,
Davao City, Philippines
Tel No.: (63 82) 221-7580 to 85
E-mail: fred@mail.uic.fapenet.org

Mr. Danto C. Alon
Farm Manager
Comval Tropical Fruit, Inc.
FSDizon Farms, Inc.
Riverview, Bacaca
Davao City, Philippines
Tel. No.: (63) 221-7580 to 85
E-mail: fred@mail.uic.fapenet.org

Mr. Romy D. Serilo
Research Head
DOST-AM Plants
Bgy. Wakat, Nagcarlan, Laguna
Mobile No.: (63) 918-860-8964
E-mail: rserilo@hotmail.com

Facilitators

Banana

Dr. Ramon V. Valmayor
Honorary Research Fellow
C / o PCARRD Headquarters
Paseo de Valmayor, Los Banos 4030
Laguna, Philippines
Tel. No.: (63-49) 536-0532
Fax No.: (63-49) 536-0578
E-mail: aspnet@laguna.net

Dr. Shin-Chuan Hwang
Director
Taiwan Banana Research Institute
Chiuju, Pingtung, Taiwan 90403
Tel. No: (886-8) 739-2113
Fax. No: (886-8) 739-3647
Email: tbri@ksts.seed.net.tw

Citrus

Dr. Florendo C. Quebral
Team Leader, Vegetable, Legumes and Rootcrops
PCARRD-DOST
Los Baños, Laguna 4030 Philippines
Tel. No.: (63-49) 536-0014 to 15
Fax. No.: (63 49) 536-0016 / 536-0132

Rapporteurs

Banana

Mr. Angelito T. Carpio

Science Research Specialist II and Commodity on Fruits
Crops Research Division
PCARRD-DOST
Los Banos, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (63 49) 536-0016 / 536-0132
E-mail: lito@ultra.pcarrd.dost.gov.ph

Ms. Lorna E. Herradura
Senior Agriculturist
Bago-Oshiro, Davao City, Philippines
Tel. No.: (62 82) 293-0107
Fax No.: (62 82) 293-0107
E-mail: banphil@dv.weblinq.com

Ms. Versalynn N. Roa
Regional Program Assistant
INIBAP-ASPNET
c/o PCARRD Headquarters
Paseo de Valmayor, Los Banos 4030
Laguna, Philippines
Tel. No.: (63-49) 536-0532
Fax No.: (63-49) 536-0578
E-mail:aspnet@laguna.net

Citrus

Ms. Guinevere I. Ortiz
Science Research Specialist II and Commodity on Fruits
Crops Research Division
PCARRD-DOST
Los Banos, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (63 49) 536-0016 / 536-0132
E-mail: giortiz@ultra.pcarrd.dost.gov.ph

Ms. Ma. Angeli G. Maghuyop
Science Research Specialist II and Commodity on Fruits
Crops Research Division
PCARRD-DOST
Los Banos, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (63 49) 536-0016 / 536-0132
E-mail: agm@ultra.pcarrd.dost.gov.ph

Mr. Celso Carreon
Agriculturist
Bureau of Plant Industry - Davao National Crop
Research and Development Center
Bago-Oshiro, Davao City, Philippines
Tel. No.: (63 82) 293-0107
Fax No.: (63 82) 293-0107

Prof. Hong-Ji Su
Professor
Department of Plant Pathology
National Taiwan University
Taipei, Taiwan
Tel. No.: 886-2-236-5717
E-mail: hjsu@ccms.ntu.cdu.tw

Organizers

INIBAP-ASPNET

Dr. Agustin B. Molina, Jr.
Regional Coordinator
C/o PCARRD Headquarters
Paseo de Valmayor, Los Banos 4030
Laguna, Philippines
Tel. No.: (63 49) 536-0532
Fax No.: (63 49) 0578
Email: aspnet@laguna.net /
gmolina@laguna.net

Dr. Ramon V. Valmayor
Honorary Research Fellow
C/o PCARRD Headquarters
Paseo de Valmayor, Los Banos 4030
Laguna, Philippines
Tel. No.: (63 49) 536-0532
Fax No.: (63 49) 536-0578
E-mail: aspnet@laguna.net

Ms. Versalynn N. Roa
Regional Program Assistant
INIBAP-ASPNET, c/o PCARRD Headquarters
Paseo de Valmayor, Los Banos 4030
Laguna, Philippines
Tel. No.: (63 49) 536-0532
Fax No.: (63 49) 536-0578
E-mail:aspnet@laguna.net

FFTC-ASPAC

Dr. Iwao Watanabe
Deputy Director
5th Flr. 14 Wenchow St.,

Taipei, Taiwan
Tel. No.: 886-2-2362-6239
Fax No.: 886-2-2362-0478
E-mail: fftc@ms1.hinet.net

Dr. Woo Nang Chang
Project Coordinator
5th Flr. 14 Wenchow St.
Taipei, Taiwan
Tel. No.: 886-2-2362-6239
Fax No.: 886-2-2362-0478
E-mail: fftc@ms1hinet.net

Ms. Jan L. Bay-Petersen
Information Officer
5th Flr. 14 Wenchow St. Taipei, Taiwan
Tel. No.: 886-2-2362-6239
Fax No.: 886-2-2362-0478
E-mail: fftc@agnet.org

National Taiwan University

Prof. Hong-Ji Su
Professor
Department of Plant Pathology
National Taiwan University
Taipei, Taiwan
Tel. No.: 886-2-236-5717
E-mail: hjsu@ccms.ntu.cdu.tw

BPI-DNCRDC

Supt. Orlando C. Pascua
Officer-in-Charge
Bago-Oshiro
Davao City, Philippines
Tel. No.: (63 82) 293-0107
Fax No.: (63 82) 293-0107

Ms. Lydia V. Magnaye
Supervising Agriculturist
Bago-Oshiro
Davao City, Philippines
Tel. No.: (63 82) 293-0107
Fax No.: (63 82) 293-0107
E-mail: banphil@dv.weblinq.com

Ms. Lorna E. Herradura
Senior Agriculturist
Bago-Oshiro, Davao City, Philippines
Tel. No.: (63 82) 293-0107
Fax No.: (63 82) 293-0107
E-mail: banphil@dv.weblinq.com

Mr. Celso Carreon
Agriculturist
Bureau of Plant Industry - Davao National Crop
Research and Development Center
Bago-Oshiro Davao City, Philippines
Tel. No.: (63 82) 293-0107
Fax No.: (63 82) 293-0107

PCARRD

Dr. Crisanto R. Escaño
Director and Scientist III
Crops Research Division
PCARRD-DOST
Los Baños, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (063-49) 536-0016 / 536-0132
E-mail:crescano@ultra.pcarrd.dost.gov.ph

Mr. Angelito T. Carpio
Science Research Specialist II and Commodity on Fruits
Crops Research Division
PCARRD-DOST
Los Banos, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (63 49) 536-0016 / 536-0132
E-mail: lito@ultra.pcarrd.dost.gov.ph

Ms. Guinevere I. Ortiz
Science Research Specialist II and Commodity on Fruits
Crops Research Division
PCARRD-DOST
Los Banos, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (63 49) 536-0016 / 536-0132
E-mail: giortiz@ultra.pcarrd.dost.gov.ph

Ms. Ma. Angeli G. Maghuyop
Science Research Specialist II and Commodity on Fruits
Crops Research Division
PCARRD-DOST
Los Banos, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (63 49) 536-0016 / 536-0132
E-mail: agm@ultra.pcarrd.dost.gov.ph

Mr. Lauro M. Lapitan
Science Research Aide
Crops Research Division
PCARRD-DOST
Los Banos, Laguna 4030 Philippines
Tel. No.: (63 49) 536-0014 to 15
Fax No.: (63 49) 536-0016 / 536-0132