# Citrus Virus and Virus-like Pathogens: A Continuing Evolution of Progress and Problems

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ABSTRACT. The International Organization of Citrus Virologists (IOCV) began in 1957 when Dr. J. M. Wallace realized the value of exchanging knowledge and research results among international citrus pathologists. Ten graft-transmissible diseases of citrus were reported at the First IOCV Conference in 1957, and the South American Continent had witnessed the destruction of millions of citrus trees due to citrus tristeza virus. Since then, great progress has been made in the identification and characterization of graft-transmissible pathogens of citrus. Contributions from IOCV members has resulted in much new knowledge of citrus diseases including those once thought to be caused by viruses which are actually caused by other agents such as viroids, spiroplasmas, phytoplasmas and a fastidious bacterium. New citrus pathogens were identified, relationships to pathogens in other crops discovered, and an array of diversity which occurs within groups of citrus pathogens were recognized and described. Great strides were made in development and application of better, sensitive and more rapid diagnostic methods. Epidemiological studies have aided more traditional approaches to provide new insights on pathogen spread and control. Control of citrus diseases has been improved by further development of clean stock/certification programs, development of new cultivars with disease resistance or tolerance, and development of improved techniques for pathogen elimination in budwood sources. Citrus virologists are often on the forefront of development and application of new biotechnological methods and the application of molecular biology. Despite all these advances, there are still several important graft-transmissible diseases of citrus which remain uncharacterized. New pathogens, including several with efficient insect vectors, continue to appear. The trends of more intensive agriculture, increased international travel and commerce, and lack of enforced quarantine regulations in many areas has increased the possibility of rapid long-range dispersal of pathogens and pests into new areas. Application of new technologies require increased investment in equipment, training, and development of better knowledge of host-pathogen systems and interactions. The role of IOCV to promote international study and dissemination of knowledge of graft-transmissible diseases of citrus is now even more important now than it was when the first Conference was held.

As the XIIIth Conference of the International Organization of Citrus Virologists (IOCV) begins in 1995, we can look back with satisfaction at the progress that has been made towards understanding and controlling graft-transmissible diseases of citrus. This experience tells us that achieving a fuller understanding and control of these diseases will require a much greater effort than ever imagined when the first Conference of IOCV was held in 1957. This brief overview is intended to highlight some of the progress made over the past 38 years, and to provide a perspective of what the future might hold for us as citrus virologists and pathologists.

The IOCV Conferences are held about every three years. At each conference, there have been several advances reported towards a better understanding of viruses and graft-transmissible diseases of citrus. Some of these advances are listed in Table 1.

"Diverse and complex" may best summarize what we have learned about graft-transmissible pathogens of citrus in these 38 years. What were once called "virus" diseases of citrus, often diagnosed by symptoms, are actually diseases caused by an array of pathogens which now include at least five viroids, a spiroplasma, a phytoplasma, a liberobacter, and at

[1]Dr. Garnsey was originally invited by the Conference Organizers to present the keynote address but was unable to attend the Conference. This invitation was extended to Dr. Lee who presented this paper. The final manuscript was prepared jointly by both authors.

TABLE 1
HIGHLIGHTS OF PREVIOUS CONFERENCES OF THE INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS (IOCV)

**Ist Conference IOCV, 1957 (California)**
- Decline, stem pitting and seedling yellows components of citrus tristeza virus (CTV) recognized
- Pathological anatomy of CTV defined
- Nucellar embryony used to obtain virus-free citrus
- Budwood certification programs developed

**IInd Conference IOCV, 1960 (Florida)**
- Graft transmission of stubborn demonstrated
- Mechanical transmission of infectious variegation virus shown
- Association of likubin with CTV proposed
- Virus-like nature of impietratura suggested
- Synergy between unrelated viruses shown

**IIIrd Conference IOCV, 1963 (Brazil)**
- CTV particles observed by electron microscopy
- Citron test for exocortis developed
- Cross-protection used to test relationship between psorosis and concave gum
- Sweet orange stem pitting recognized as production problem
- Association of xyloporosis and cachexia studies
- Budunion creases associated with virus-like agents

**IVth Conference IOCV, 1966 (Italy)**
- Psyllids recognized as vectors of huanglongbing (HLB)
- Relationship between *HLB*, leaf mottling and likubin suggested
- Citrange stunt and tatterleaf recognized
- Cristacortis recognized
- Purification of crinkly leaf virus reported
- Fluorescent markers used for rapid detection of HLB

**Vth Conference IOCV, 1969 (Japan)**
- Beneficial use of tree dwarfing suggested
- Exocortis transmitted to *Gynura*
- Knife-cut transmission of exocortis demonstrated
- A new decline in Brazil reported (decliñio)
- Epidemiology of HLB clarified and strains identified
- Seedling yellows recovery reported

**VIth Conference IOCV, 1972 (Swaziland)**
- *Spiroplasma citri* cultured
- "walled" mycoplasma-like bacterium associated with HLB
- Purification and serology of citrus variegation virus advanced
- Increased mechanical transmission to citrus viruses to herbaceous plants
- Chemotherapy of HLB demonstrated
- EM used for rapid detection of CTV in eradication program

**VIIth Conference IOCV, 1975 (Greece)**
- Viroid nature of exocortis established
- Multiplication of *S. citri* in leafhoppers demonstrated
- Seed transmission of psorosis reported
- Shoot-tip grafting used in certification programs

**VIIIth Conference IOCV, 1979 (Australia)**
- Serological detection of CTV
- CTV inclusion bodies recognized
- Biological control of psyllid vectors of HLB
- Mechanical transmission of citrus ringspot

TABLE 1 (CONTINUED)
HIGHLIGHTS OF PREVIOUS CONFERENCES OF THE INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS (IOCV)

**IXth Conference IOCV, 1983 (Argentina)**
- Slash-cut inoculation of CTV to citrus relatives
- Stubborn vectors and epidemiology defined
- Natural spread of psorosis in Argentina reported
- Graft transmission of leprosis
- dsRNA analysis for CTV
- PAGE detection for viroids

**Xth Conference IOCV, 1986 (Spain)**
- Monoclonal antibodies for CTV developed
- Viroid etiology of cachexia confirmed
- Discovery of witches broom of Mexican lime in Oman
- Dual components of citrus ringspot discovered
- Viburnam identified as alternate host for satsuma dwarf

**XIth Conference IOCV, 1989 (Florida)**
- Complexity of citrus viroid complex expanded
- Cross protection between viroids reported
- Transmission of blight by root grafting reported
- Characterization of vein enation as probable luteovirus
- Serological detection of HLB
- Standardized biocharacterization of CTV
- Strain-selective antibody developed for CTV

**XIIth Conference IOCV, 1992 (India)**
- Severe sub-isolates of CTV recovered from mild parent source
- Serological detection of citrus blight reported
- *Xylella fastidiosa* associated with citrus variegated chlorosis
- ELISA used to study CTV epidemiology
- Citrus ringspot designated a spirovirus

**XIIIth Conference IOCV, 1995 (China)**
- Discovery of DI-RNAs associated with CTV infection
- CTV diagnosis based on sequence information developed
- Biological control of citrus aphids enhanced
- Indian citrus mosaic associated with a badnavirus
- Characterization of leprosis virus
- Citrus chlorotic dwarf, a new whitefly-transmitted virus-like disease reported
- Detection of HLB by PCR

least six different groups of viruses (Table 2). As each pathogen or group of pathogens has been studied, the variability and diversity became even more apparent. Alarmingly, many additional and previously unrecognized pathogens have been discovered. Some of these pathogens may cause severe symptoms on citrus and have efficient insect vectors. In some instances, the pathogens are not limited to citrus and affect other crops as well.

At one time, symptoms were instrumental in the identification of virus and graft-transmissible diseases of citrus. This actually limited the study and control these diseases. Now many of the important pathogens of citrus, including citrus tristeza virus (CTV), tatterleaf, satsuma dwarf, citrus variegation and citrus leaf rugose viruses, citrus viroids, and *Spiroplasma citri* have been extensively characterized and partially or completely sequenced. Detection methods have been developed which utilize pathogen-specific antibodies which are sensitive and adaptable to large-scale usage. Poly-

TABLE 2
VIRUS AND VIRUS-LIKE DISEASES RECOGNIZED IN 1957 AT THE FIRST CONFERENCE OF INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS COMPARED TO THIS INFORMATION AT THE THIRTEENTH CONFERENCE HELD IN 1995

| Disease | Pathogen group |
|---|---|
| Ist Conference (1957) | |
| • Psorosis | |
| • CTV | |
| • Stubborn | |
| • Satsuma dwarf | |
| • Xyloporosis | |
| • Exocortis | |
| • Vein enation | |
| 13th Conference (1995) | |
| "Characterized" viruses: | |
| • Citrus leaf rugose | Ilarvirus group |
| • Citrus mosaic | Badnavirus group |
| • Citrus ringspot | Spirovirus group |
| • Leprosis | Rhabdovirus group |
| • Satsuma dwarf | ? group |
| • CTV | Closterovirus group |
| • Tatterleaf | Capillovirus group |
| • Vein enation | Luteovirus group |
| Viroids: | |
| • CEV (exocortis) | |
| • CVd Ia | |
| • CVd Ib | |
| • CVd IIa | |
| • CVd IIb (cachexia) | |
| • CVd IIc | |
| • CVd IIIa | |
| • CVd IIIb (dwarfing) | |
| • CVd IIIc | |
| • CVd IIId | |
| • CVd IV | |
| • Gummy bark | |
| • Gum pocket | |
| Prokaryotes: | |
| • Asian HLB | "*Candidatus* Liberobacter asiaticum" |
| • African HLB | "*Candidatus* L. africanum" |
| • Stubborn | *Spiroplasma citri* |
| • Witches broom of lime | "*Candidatus* Phytoplasma aurantifolia" |
| • Citrus variegated chlorosis | |
| "Uncharacterized " virus-like diseases: | |
| • Algerian navel orange "virus" | |
| • Brittle twig yellows | |
| • Budunion crease | |
| • Citrus yellow mottle | |
| • Citrus chlorotic dwarf | |
| • Concave gum | |
| • Cristacortis | |
| • Fatal yellows | |
| • Leathery leaf | |
| • Impietratura | |
| • Popcorn psorosis | |

TABLE 2 (CONTINUED)
VIRUS AND VIRUS-LIKE DISEASES RECOGNIZED IN 1957 AT THE FIRST CONFERENCE OF INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS COMPARED TO THIS INFORMATION AT THE THIRTEENTH CONFERENCE HELD IN 1995

| Disease | Pathogen group |
|---|---|
| • Rubbery wood | |
| • Zonate chlorosis | |
| • Yellow vein | |
| • Measles | |
| Declines of unknown etiology: | |
| • Citrus blight | |
| • Australian citrus dieback | |

merase chain reaction and nucleic acid hybridization assays have been applied for highly sensitive detection and diagnosis. Application of these sensitive detection methods to the study of several pathogens has brought a new appreciation for the genetic diversity which can exist at the pathogen level.

While new methods of pathogen detection which promise greater sensitivity, specificity, and efficiency are evolving rapidly, it may be difficult and expensive to incorporate these techniques into existing testing programs. Progress needs to be made on the development and acceptance of international standards for indexing, certification, and for movement of pathogen-free citrus germplasm. Exchange of information, training and the ability to procure essential equipment are needed to aid the incorporation of new and better detection methods to ultimately achieve better control of the citrus diseases.

A great deal has been learned of the insect vectors of CTV, huanglongbing (HLB) (greening), and stubborn. Unfortunately, it has been realized that man has been an effective vector of graft-transmissible citrus pathogens, especially over long distances. Costly disease epidemics have been created by the long-distance movement of infected or infested citrus plants and budwood.

The ability to develop virus-free propagating material, along with the development of schemes to ensure that virus-free plants are available for use in field plantings, has been the biggest contribution towards the control of graft-transmissible citrus pathogens. This was made possible by the development of shoot-tip grafting and thermotherapy procedures, combined with improved indexing techniques. Expanded application of certification programs can undoubtedly prevent many disasters in the future.

While much effort has been made to avoid or eliminate citrus virus and graft-transmissible pathogens in propagating materials, it has been discovered that some pathogens may be useful, at least in special circumstances. For example, mild strains of CTV are now used to reduce stem pitting damage of citrus scions in some countries. Viroids can be selected which reduce tree size and enable high density plantings of smaller trees. Genes taken from citrus viruses are now being used to genetically engineer transgenic plants which should result in enhanced virus resistance.

While progress in the molecular characterization of viruses can result in the development of more sensitive detection methods and better control methods, the fundamental knowledge gained often tells us, as scientists, how complex these systems are and how much more we need to learn. For example, recent work on the molecular characterization and comparisons of CTV strains is showing unexpected diversity among and within isolates. This may suggest the presence of several strains within a given isolate, thus raising the question as to which strain or strains is the one causing the damage or producing symptoms. Which strain should be targeted when trying to select a mild strain for cross-protection, or do the strains have to be mixed to achieve cross-protection? When developing genetic engineering methods to produce virus resistant transgenic citrus plants, which strain or strains should be targeted? The genetic diversity of viruses also presents potential problems when using methods such as reverse transcriptase-polymerase chain reaction-based assays for molecular characterization. Non-representative or "hitch hiker" strains may inadvertently be selected and focused upon, rather than the true disease-causing strain(s). However, other molecular methods are available to minimize or prevent this possibility.

While impressive progress has been made on the characterization of some graft-transmissible pathogens, there are other pathogens of citrus which remain uncharacterized except for the fact that they are graft-transmissible and, therefore, infectious. Psorosis was the first virus-like disease to be described, yet the psorosis complex remains one of the most poorly understood groups of virus-like pathogens. Citrus blight was first described over 100 years ago and has been a destructive disease in many of the largest citrus-producing areas of the world; yet, the causal agent of blight remains elusive. Much has been learned about the causal agent of HLB, one of the most devastating diseases of citrus, but the causal bacterium remains uncultured and, thus, poorly characterized. The soil-borne vector of satsuma dwarf also remains undetermined.

It is alarming that new, destructive diseases of citrus continue to appear and spread. Ten years ago the citrus variegated chlorosis (CVC) disease was unknown. We now know it is caused by a pathotype of *Xylella fastidiosa*, a xylem-inhabiting bacterium, and that it is spread by several species of sharpshooter vectors as well as by spread through infected nursery stock. This disease may have moved into citrus from coffee. Today, CVC is present in all citrus-producing areas of Brazil and is removing citrus groves from production in the northern part of São Paulo State. A new whitefly-transmitted virus-like pathogen has been reported in Turkey, and this disease is increasing in importance. A phytoplasma, spread by an insect vector, threatens continued production of acid limes in the Arabic Peninsula. The spread of the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), northward throughout the Caribbean Basin from South America threatens the continued production of about 180 million citrus planted on sour orange rootstock throughout the region, due to the effective spread of decline strains of CTV. The vector of HLB, *Diaphorina citri* Kuwayama, is migrating northward from the southern part of the South American Continent, providing the means to spread HLB should some unsuspecting person smuggle this destructive citrus disease into the Americas.

For the foreseeable future, various disease management strategies must be used to protect susceptible cultivars. For some diseases, this may require better application of existing technologies, such as use of virus-free budwood. For other diseases, it will require better knowledge of the host-pathogen-vector interactions involved and innovative use of new approaches. Regional and even global approaches may be required rather than the local programs of the past, especially in the face of increased international trade. Citrus virologists cannot work in isolation, but multi-disciplinary teams and partnerships must be developed. The social impact of the loss of citrus production due to destructive citrus diseases must be recognized and reduced in areas where small farmers grow citrus as a part of their sustainable agricultural system.

Development of improved host tolerance or resistance is often seen as the panacea to control many existing virus and graft-transmissible pathogens and as a means to decrease our current genetic vulnerability to new problems that will arise. However, even with rapid advances in genetic engineering, this task will be difficult and may be achievable only with much higher levels of research. Multiple diseases, sources of resistance which are poorly defined, and years required to assess horticultural characteristics are problems to deal with, even after a source of resistance is found and incorporated.

## WHAT ROLES WILL IOCV PLAY IN THE FUTURE?

Citrus is recognized as a valuable international commodity. As such, it should not be surprising that the current state of knowledge on viruses and graft-transmissible diseases of citrus has been developed as the result of extensive international cooperation and interaction between scientists who often have different, but complementary, backgrounds and training. IOCV has played a very important role in developing international cooperation among citrus scientists. IOCV must continue this important role. Our IOCV conferences provide forums for personal contact among citrus scientists, and to establish bonds that are essential for cooperation. The IOCV Newsletter provides a means to share observations among scientists between conferences, and for members to keep up to date on events happening in the area of citrus virology and pathology. In the past, IOCV has played significant roles in preparation of manuals for indexing protocols, developing methods for safe international movement of citrus germplasm, and making available slide sets to aid in field diagnosis and in teaching.

## WHAT CAN IOCV DO TO CONTINUE THE USEFUL ROLES IT HAS FULFILLED SINCE 1957?

IOCV has successfully used conferences, published proceedings, sponsored books and manuals, and published newsletters to promote international study and dissemination of knowledge for control of graft-transmissible diseases of citrus. The recent development of electronic mail, the logarithmic growth of the internet as a means to establish interactive forums and chat rooms and with the use of web-pages to keep information current and up to date, and availability of computers with CD-ROM drives for persons/laboratories not having internet access all offer new possibilities to IOCV which are limited only by our imagination. Just as virus diseases have interactions with the host and insect vectors, there is a need for us to have interactions with horticulturalists, entomologists, and nuserymen to work together to achieve control of many of these diseases. Much has been done, but obviously more complex and more difficult tasks lie ahead. The XIIIth Conference of IOCV presents a special opportunity for many delegates from other countries to visit and appreciate China, the ancient home of citriculture. It also presents an excellent opportunity to pause, examine the path we have followed, and ponder together how we can jointly meet these challenges in the exciting future we will share.

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