SILVER ANNIVERSARY

Twenty-Five Years of Outstanding Progress in Citrus Virus Research Marks IOCV's Silver Anniversary

S. M. Garnsey

ABSTRACT. Remarkable progress has been made on important citrus virus and viruslike pathogens in the past 25 years, much of it through outstanding efforts of IOCV members. The causal agents of greening, stubborn, exocortis, and tristeza have been characterized. Identification and characterization of the pathogens causing these major diseases have greatly improved control strategies and advanced basic knowledge on viroids, spiroplasmas and fastidious bacteria as plant pathogens. A number of new virus and viruslike diseases have been described, as well as new variants of known ones. Detection procedures for many viruses have been improved by use of better indicator plants and by new transmission, purification and serology techniques. Shoot-tip grafting and heat therapy have accelerated production of virus-free scion sources of major varieties and facilitated budwood certification. Isolates of tristeza and exocortis have been used beneficially for cross protection and tree dwarfing, respectively. Many challenges remain, but the nine conferences of IOCV and eight volumes of IOCV Proceedings have created a strong framework for worldwide communications and coopeartion to continue research.

The 9th Conference of the International Organization of Citrus Virologists (IOCV) marks its 25th anniversary. The past 25 years have witnessed dramatic progress in characterization and control of citrus virus and viruslike diseases (CVVLD) and much of this progress can be credited to IOCV and to its members.

As we enter the next quarter century of IOCV activity, it is appropriate to examine how we have improved our knowledge and understanding of CVVLD and have utilized this knowledge to control these diseases. At the same time, it is a good opportunity to recognize voids in our knowledge and needs for new research.

Much of the extensive progress made on CVVLD problems can be credited to the exchange of information and ideas and the development of joint research efforts fostered by IOCV. The eight previous IOCV conferences brought scientists together from many different regions and countries, and with the associated pre- and/or postconference activities have taken IOCV delegates to at least 15 different countries (fig. 1). Formal exchange of information has occurred through presentation of Proceedings. The eight IOCV Proceedings published to date contain 439 papers and 2228 pages.

Study programs and tours associated with each conference have provided delegates invaluable opportunities to study disease conditions in different areas. These firsthand observations augment information in formal papers, and have clarified many previous misconceptions or misunderstandings. Delegates have also observed research experiments and facilities at each meeting site, and also have interacted with the scientific support staff in each location.

Personal interchange also has been fostered by IOCV, and many of us first became acquainted at IOCV conferences. These acquaint-

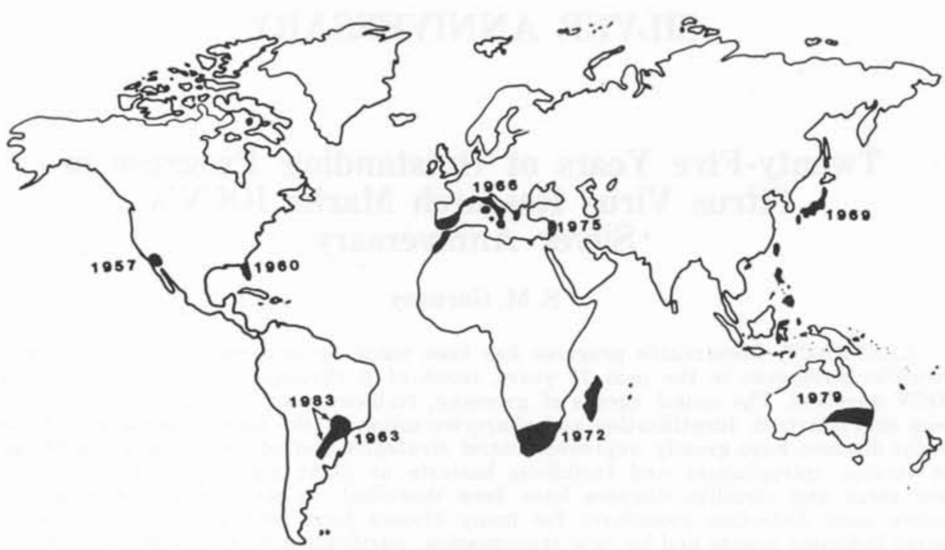

Fig. 1. Sites for the first nine IOCV conferences and associated conference programs are shown by shaded area and date.

ances have often blossomed into permanent friendships and collaborative research efforts which span the globe. The benefits from these associations are intangible and often difficult to document, but surely are a valuable legacy of the first 25 years of IOCV and one desired by the organizer of the first IOCV conference and our first chairman, Dr. J. M. Wallace.

This paper is not a comprehensive summation of all work on virus and viruslike problems over the past 25 years, but highlights some of the most significant accomplishments especially pertinent to IOCV and its members.

CHARACTERIZATION OF CITRUS VIRUS AND VIRUSLIKE PATHOGENS

When the first Conference was held in 1957, numerous citrus diseases had been ascribed to virus or viruslike pathogens, but the nature of the causal agents was unknown. Viral etiology was assumed based on symptoms, the infectious nature of the diseases, and lack of association with bacterial or fungal pathogens. No citrus virus had been sap transmitted experimentally and none had been visualized in the electron microscope.

Outstanding progress has been made in the past 25 years. Some landmark contributions included visualization of the particle of citrus tristeza virus (CTV), discovery and culture of the spiroplasma causing stubborn disease, discovery of the procaryote causing greening disease, and discovery that exocortis is caused by a viroid. These pioneer advances reflect unusual insights, perseverance, and hard work under difficult circumstances. Charazterization of citrus pathogens often has come through the accumulated efforts of several workers over an extended period. For example, scientists in several laboratories in different countries have contributed to characterization of the CTV particle, production of CTV-specific antisera, development of rapid, sensitive detection techniques and new approaches to CTV control. The 25-year progress in the mechanical transmission, electron microscopy, purification and serology of 16 citrus virus and viruslike pathogens is summarized in Table 1. Although extensive progress can be seen with

TABLE 1
PROGRESS IN THE CHARACTERIZATION OF SOME CAUSAL AGENTS OF VIRUS AND VIRUSLIKE DISEASES OF CITRUS

Disease	Mech. trans.		E.M.		Purification		Serology	
	1957	1982	1957	1982	1957	1982	1957	1982
Tristeza	—	yes	—	yes	—	yes	—	yes
Stubborn	—	—	—	yes	—	yes*	—	yes
Greening	—	?	—	yes	—	—	—	—
Exocortis	—	yes	—	yes	—	yes	—	—
Infection variegation	—	yes	—	yes	—	yes	—	yes
Crinkly leaf	—	yes	—	yes	—	yes	—	yes
Leaf rugose†	—	yes	—	yes	—	yes	—	yes
Satsuma Dwarf	—	yes	—	yes	—	yes	—	yes
Tatterleaf†	—	yes	—	yes	—	?	—	—
Ringspot†	—	yes	—	—	—	—	—	—
Psorosis	—	yes	—	—	—	—	—	—
Xyloporosis	—	yes	—	—	—	—	—	—
Impietratura	—	—	—	—	—	—	—	—
Cristacortis†	—	—	—	—	—	—	—	—
Vein enation	—	—	—	yes	—	—	—	—
Leprosis	—	—	—	yes	—	—	—	—

*Cultured.
†Not described in 1957.

a number of pathogens, little progress has been made with some others including two of those recognized earliest, psorosis and xyloporosis.

TRANSMISSION

Significant new information on natural spread of CVVLD has also been developed in the past 25 years. By 1957, the aphid transmission of CTV was well recognized, but little was known about the natural spread of other pathogens. Since then two psyllid vectors of greening, several leaf-hopper vectors of stubborn, transmission of CEV on knife blades, and seed transmission of psorosis in trifoliate orange and citranges have been discovered.

Progress on transmission has also come from input by a number of individuals over a period of time. Team efforts and international cooperation are especially evident for the work on greening and stubborn. Improved knowledge of pathogen-vector relationships and of pathogen host range have contributed markedly to development of control strategies for stubborn and greening.

Man still remains a prime vector of CVVLD, especially over long distances, and many serious disease outbreaks can be traced to importation and spread by subsequent propagation and/or vectors.

HOST INTERACTION

During the past 25 years, our understanding of pathogen-host interactions with CVVLD has greatly improved. Prior to 1957, classic cytological studies had revealed some effects of CTV on citrus hosts, and strain differences in pathogenicity have been observed for several viruses, including CTV.

Most of our progress in studying host-pathogen interactions, however, is based on our recent ability to obtain pure cultures of individual pathogens. Early work with mixed infections often resulted in confusion about the specific association of disease symptoms and a pathogen. Several forms of greening disease were initially associated with CTV infection because affected plants were doubly infected, and some relationships in the psorosis group established by cross-protection tests were later shown to be erroneous because of contamination. Subsequently, mechanical transmission, purification, vector transmission, differential host resistance, and differential responses to therapy techniques have all been useful singly or in combination to obtain pure cultures of many CVVLD. Lack of pure cultures still hampers identification of specific symptoms and relationships with the psorosis complex, cristacortis, and impietratura, among others.

Studies on mechanical transmission, purification, and serology have improved our ability to quantitate virus accumulation within the host and to determine pathogen distribution. We have learned that virus replication is a dynamic process, and that virus titer can fluctuate greatly according to host species, tissue age and environmental conditions. Early workers concluded that citrus viruses occurred only in low concentrations within citrus hosts. Subsequently, we have learned that even some tissue-restricted viruses such as CTV can occur in relatively high concentration, and several citrus viruses are routinely purified directly from citrus.

Although citrus plants are systemically affected by stubborn, by greening, and by various viruses, the distribution of the pathogens may be highly irregular. It often is difficult to recover or transmit a given pathogen from substantial portions of an infected plant. The pathogens causing stubborn, greening, and CTV are all strongly phloem-associated. The tatterleaf-citrange stunt virus (TL-CSV) and citrus ringspot virus (CRSV), often invade and infect only portions of a host in an irregular pattern.

The propagation of commercial citrus trees as grafted combinations creates a special site for viral reaction—the graft union. As classically demonstrated with CTV, a rootstock variety and a scion variety each separately tolerant of CTV may form a highly CTV-intolerant combination when grafted together. A number of interesting stock/scion-associated problems have been observed which have not been well characterized, but some are apparently due to infectious agents.

The interaction of citrus viruses with their hosts on a molecular level has not been well studied. By-products of infection have been analyzed for diagnosis, but more direct studies are lacking. The single-stranded RNAs of some citrus viruses apparently function as messenger RNAs to alter certain cell functions. Some symptoms, however, apparently are indirect responses to pathogen action. A unique situation that awaits clarification is how the small RNA molecule of citrus exocortis viroid (CEV) functions to direct its replication and cause symptoms.

DETECTION

Citrus indicators. In 1957, identification of citrus virus and viruslike diseases was based entirely on symptoms in field trees or graft-inoculated citrus indicators, and the discovery that CTV infections could be diagnosed accurately and relatively rapidly on Mexican lime indicators had been the major advance. Since 1957, further improvement in utilization of citrus indicators has occurred. An outstanding example is the Etrog citron test for CEV. Sensitive indicators have been described for a number of CVVLD; however, in some cases symptoms are not highly specific or rapidly formed. Symptoms in the wood do not appear rapidly, and diagnosis of a specific pathogen in field trees with multiple infections by graft inoculation to citrus indicators is still difficult. Citrus hosts remain valuable for characterizing severity of strains of a given pathogen.

The need for healthy, vigorous plants and appropriate growing conditions has been recognized. Mild temperature conditions favor detection of many viruses, but warm temperatures are required for CEV, stubborn, and xyloporosis. Careful selection of inoculum tissue has improved detection of some irregularly distributed pathogens such as stubborn, greening, and citrus ringspot. Novel grafting techniques have been developed to improve transmission.

Noncitrus indicators. As information on the mechanical transmissibility of citrus viruses has increased, indexing procedures based on mechanical (sap) inoculation to herbaceous plants have been detected in noncitrus hosts by mechanical inoculation. These procedures are now commonly used for research and for some routine indexing. Careful selection of inoculum, appropriate plants, and good conditions are required. When these conditions are met, indexing periods can be reduced from months to a few days. Less time is needed to grow indicator plants, and viruses not detected by currently used citrus indicators may be revealed.

Biochemical tests. Several indexing procedures are based on identification of specific marker substances produced as a result of CVVLD infection. The most succesful has probably been the fluorescent marker test for greening based on presence of a gentisoyl glucoside. Difficulties in proving specificity of these procedures plus equipment and technique requirements have limited general applications.

Microscopy. Light microscopy of cytological changes induced by CTV infection was used prior to 1957 for diagnosis. Light microscopy has been used more recently to identify viral-specific inclusion bodies, and to locate procaryotic pathogens. Availability of fluorescent-labeled antibodies has also enhanced detection of specific pathogens by light microscopy.

The observation of unusual filamentous particles in extracts from CTV-infected plants by electron microscopy (E.M.) led to the use of E.M. for diagnosis of CTV. A more sensitive modification, serologically (immuno) specific E.M. has made E.M. more attractive for detection of viruses to which specific antisera have been prepared. Microscopy is the most rapid detection procedure for single samples of some pathogens (table 2).

Serology. The greatest advances in detection of CVVLD have occurred through application of serology. Immunodiffusion procedures were developed for citrus leaf rugose virus (CLRV), Citrus variegation virus (CVV), and CTV, but required good sources of tissue for reliable results and sizable quantities of antisera for extensive use. Application of the enzyme-linked immunosorbent assay (ELISA) procedures to CTV detection provided a sensitive and efficient procedure, well adapted to large-scale use. In the past 5 years, ELISA has been used extensively in many countries for CTV detection and ELISA has been used also for stubborn, CVV, CLRV, satsuma dwarf virus, and citrus mosaic virus.

Development of serological procedures resulted from research on characterization and purification. In turn, serological assays provided