14

BARD

FINAL REPORT
PROJECT NO. US-2346-93

Citrus Tristeza Virus: Molecular Approaches to Cross Protection

**W.O. Dawson, C.L. Niblett, R.F. Lee, M. Bar-Joseph, R. Gafny,
M. Mawassi**

1998

TABLE OF CONTENTS

Cover page.....	1
Table of contents.....	2
I. Abstract.....	3
II. The original objectives.....	4-5
III. Relevant background, methodology, results, and discussion.....	5-48
III. 1. Complete sequence of the CTV genome.....	11-21
III. 2. Assembly of the full-length infectious cDNA copies of the CTV genome.....	22
III. 3. Development of the protoplast assay system.....	23-29
III. 4. Defective RNAs.....	30-40
III. 5. Striking isolate diversity of the CTV isolates.....	41-48
IV. Description of the cooperation.....	49-50
V. Evaluation of the research achievements.....	51-52
VI. Conclusions.....	53
VII. List of publications.....	54-55

630.72

BAR/CT

2nd copy

הספריה המרכזית
למדעי החקלאות
בית דגן

I. ABSTRACT

Citrus tristeza virus (CTV) has the largest genomes among RNA viruses of plants. The 19,296-nt CTV genome codes for eleven open reading frames (ORFs) and can produce at least 19 protein products ranging in size from 6 to 401 kDa. The complex biology of CTV results in an unusual composition of CTV-specific RNAs in infected plants which includes multiple defective RNAs and mixed infections. The complex structure of CTV populations poses special problems for diagnosis, strain differentiation, and studies of pathogenesis. A manipulatable genetic system with the full-length cDNA copy of the CTV genome has been created which allows direct studies of various aspects of the CTV biology and pathology. This genetic system is being used to identify determinants of the decline and stem-pitting disease syndromes, as well as determinants responsible for aphid transmission.

II. THE ORIGINAL OBJECTIVES

The main original objective was to develop a genetic system to allow mapping characteristics of CTV important to developing "effective" cross-protecting isolates. It was likely that a specific cross protecting isolate would have to be designed and built for each challenging isolate threatening specific varieties of citrus in different areas. Since CTV isolates do not cross protect against all other isolates, the prevailing thought was that mild isolates that are the most genetically similar to the challenging severe isolate should be used. The next objective was to create a mild isolate from the severe challenging isolates by substituting portions of the genome that are involved in causing disease. It was hypothesized, that it should be possible to create a progeny isolate that causes no disease in the challenged citrus variety as we already have done with tobacco mosaic virus (TMV) in tobacco.

A set of sub-objectives which had to be addressed during the creation of the CTV genetic system addressed both epidemiological and pathological aspects. Specifically, the genetic heterogeneity of the CTV isolates was to be addressed, with its relationship to the cross-protective potential of "mild" CTV isolates. The success of the application of the CTV genetic system to the creation of the cross-protective isolate was from the very beginning dependent on how well the epidemiological aspects of the CTV infection would be understood.

Here is the list of the specific objectives from the original proposal.

Specific Objectives:

- 1) Assemble multiple full-genomic cDNA clones of CTV;
- 2) Develop methods to infect plants from the cDNA clones of CTV;
 - a) *in vitro* RNA transcripts

- b) *Agrobacterium tumefaciens* Ti plasmid transformation and inoculation
- c) inoculation of protoplasts with RNA and DNA
- 3) Characterize the resulting uniform population isolate of CTV;
- 4) Create and analyze CTV hybrids to map determinants of severe decline;
- 5) Create and analyze CTV hybrids to map determinants of stem pitting;
- 6) Analyze CTV hybrids to map determinants of aphid transmission;
- 7) Develop isolates of CTV that are "ideal" for cross protection;
 - a) Develop isolates of CTV to test for protection against stem pitting;
 - b) Develop isolates of CTV to test for protection against quick decline; and,
 - c) Test for ability of these mutants to cross-protect against diverse naturally occurring isolates.

III. RELEVANT BACKGROUND, METHODOLOGY, RESULTS, AND DISCUSSION.

Citrus is a major world food crop in terms of nutrition, generation of income, foreign exchange, and employment. Yearly world production is approximately \$10 billion. Citrus is a major component of both the United States and Israeli economies, with the US producing 18% of the world's citrus. Grapefruit, Oroblanco and other varieties are the largest agricultural export commodity from Israel. Citrus tristeza virus (CTV) causes serious diseases of citrus throughout the world that limit production by causing decline of trees on sour orange rootstocks and stem-pitting that debilitates trees and reduces fruit yield, size, and quality regardless of rootstock. However, recent events have greatly increased the economic importance of CTV in citrus in both the US and Israel (Lee *et al.*, 1995; Rocha-Pena *et al.*, 1995). In the Central Valley of California, the virus has

escaped the quarantine and eradication program and is spreading. In Israel and California, grapefruit, and particularly Oroblanco, are unusually susceptible to stem pitting isolates. In Florida, the efficient CTV vector, the brown citrus aphid (*Toxoptera citricida*), which had been threatening the US borders, arrived and is already endemic in commercial citrus. The 25 million trees on sour orange rootstocks are likely to be quickly lost to decline, but the majority of commercial citrus is on rootstocks resistant to decline. However, the greater threat is from stem pitting isolates. Stem pitting isolates already have been found in natural populations in Florida. These isolates are expected to begin moving into susceptible varieties, especially grapefruit, and reduce production and quality. The aphid already is in the prime grapefruit growing area. CTV will most probably not kill all trees, but undoubtably will cause losses in yields and fruit quality and impose major difficulties on the economy of citrus production in the US and Israel.

Diseases attributed to CTV vary considerably, depending on the virus isolate or population, the citrus variety and rootstock, and its interaction with aphid vectors and the climate. The most severe diseases are decline of commercial varieties that are grafted on sour orange rootstocks and stem pitting which is independent of rootstock used. Within each of these categories, symptoms can vary considerably in the severity and the rate of development (Bar-Joseph *et al*, 1979, 1989; Garnsey *et al*, 1987). Decline results in death that can occur within one growing season (quick decline) or gradually. Stem pitting results in lack of vigor and reduced productivity and quality. However, some isolates of CTV cause no noticeable effect on most commercial citrus cultivars. Decline strains of CTV caused the loss of millions of trees in most citrus growing areas. Although producing less dramatic symptoms, stem pitting strains ultimately cause greater damage to citrus production (Lee *et al*, 1992; Roistacher & Moreno, 1991). The natural spread of stem pitting strains of CTV

in Israel and the US presents a serious threat to continued culture of grapefruit, limes, and vigorous sweet orange varieties.

In Israel, CTV was introduced through importation of infected varieties during the 1920-1930s, but it was not until the early 1970s that natural spread of CTV was observed. Because much of the citrus in Israel is on sour orange rootstocks and thus very susceptible to decline, an eradication program was implemented. In spite of concerted efforts to eliminate infected trees, the virus eventually became widespread in the coastal plain area by the late 1980s. A suppression program is presently being conducted in the inland peripheral citrus regions. Remaining trees on sour orange rootstocks are at immediate risk, and stem pitting strains of CTV threaten continued production of especially red grapefruit.

CTV has long, flexuous filamentous particles *ca.* 2,000 nm long and 11 nm in diameter which are composed of a major species of 25-kDa coat protein and one molecule of single-stranded (ss), positive-sense RNA of 19,296 nt. The virus is aphid-transmissible in a semi-persistent manner by several species of aphids. The brown citrus aphid (*Toxoptera citricida*) is the most efficient vector. The brown citrus aphid is prevalent in tropical regions, while in more temperate citrus-growing areas the main vector of CTV is the melon aphid *Aphis gossypii*. The biology of CTV is very complex. The virus persists for years in a perennial host which is often exposed to multiple infections in the same host tree by both insect inoculation and grafting of the infected budwood. Infected trees are grown under different environmental conditions and infection occurs in a broad range of hosts grafted onto different rootstocks. Some of these factors probably contribute to the development of great natural diversity of CTV strains or isolates.

CTV is currently classified into the family *Closteroviridae*. There are two distinctive

characteristics of closteroviruses; association of the virus with the phloem tissue (in some cases restriction to the phloem), and a unique cytopathology featuring unusual membranous vesicles in infected cells. These vesicles are referred to as BYV-type vesicles as they were first demonstrated for the type member of the closterovirus group, beet yellows virus (BYV).

CTV now threatens the citrus industry in both California and Florida, although for different reasons. In the Central Valley of California, the primary disease management strategy has been quarantine and suppression, but in spite of this, the virus is spreading and there is an increase in the severity of the virus populations. In Florida, economic losses due to CTV have been occurring for the past 20 years, but the lack of an efficient insect vector has allowed the industry to survive. However, the brown citrus aphid arrived in Dade County in November 1995, has already spread into southern commercial areas, and is expected to be established throughout the Florida industry by the beginning of 1997. Strains of CTV causing decline of trees on sour orange rootstock are already widespread throughout the state. About 20% of the industry (20 million trees) remain on sour orange rootstock, and it is estimated that these trees will be damaged within five years. However, the rest of the Florida industry is threatened by stem pitting strains of CTV which would greatly reduce yield and fruit quality. Especially at risk is the Florida grapefruit industry as strains of CTV which cause stem pitting on grapefruit have been identified in the state by biological indexing. If this aphid is able to move to Texas, Arizona, or California, it would represent an even greater threat to US production.

The good news is that technology now is available to manage these diseases. For the next generation of trees, the strategy is to develop virus-resistant varieties. Traditional breeding has not been effective in producing virus-resistant citrus, primarily because sweet orange and grapefruit

varieties are complex hybrids of unknown origin that have not been reproduced by sexual crosses. However, several strategies of transforming plants with viral sequences or using natural resistance genes have been shown to be effective in other plant species and should also work in citrus. Agrotransformation and bombardment of citrus varieties is ongoing to produce a new generation of CTV-resistant citrus. However, the earliest realistic time that this would affect citrus production is approximately 20 years from now. Because trees can be productive up to 100 years, the value of standing trees is extraordinarily high. The strategy to reduce losses in existing plantings is to develop inocula for mild-strain cross protection, modification of strategies that have been effective against stem-pitting strains of CTV in South America, South Africa, and Australia.

The bad news is that the most promising strategies must be targeted against specific CTV sequences. Both “RNA-mediated resistance” (which now appears to be the mechanism of much of the resistance that was thought to be due to “coat-protein-mediated resistance” or “replicase-mediated resistance”) and mild strain cross-protection, work only against closely related isolates with very similar sequences. Most isolates of CTV consist of complex populations containing components often sufficiently diverse in sequence to escape these types of control. Also, many natural resistance genes against CTV in citrus relatives that potentially could be isolated and transformed into citrus are ineffective against some CTV isolates. Thus, all of these strategies must be targeted against those components of the population that cause disease. In fact, targeting the wrong components could cause changes in population structures that would intensify rather than prevent disease. Thus, the most important objective at this time is to identify the enemy. We need to know what components of the CTV population cause disease. An equally important need is to have methods to differentiate dangerous isolates of CTV from innocuous isolates of CTV as they

spread within Israel and the US.

All of the practical questions can be relatively easily addressed with the use of a manipulatable genetic system utilizing a full-length, infectious copy of the CTV genome. By substituting genome segments between CTV isolates which differ in symptomatology and vector transmission characteristics, determinants responsible for major symptoms and aphid transmission can be found. Conversely, replacement of the undesirable fragment of the virus genome with the “harmless” one will provide an ideal cross-protecting CTV isolate, which may be used for suppression of the spread of severe isolates. Identification of symptom determinants will make it possible to differentiate undesirable CTV isolates, and thus ignore harmless ones. Thus, the primary objective of the project was creation of a genetic system suitable for manipulations with the CTV genome.

This general objective was pursued *via* three directions: cloning and sequencing of the CTV genome for isolate T36, upon completion of the sequence - assembly of the full-length cDNA clones of the CTV genome, and development of the protoplast assay system to monitor CTV infectivity. To be able to use this genetic system for the outlined purposes several secondary objectives were addressed. We performed cloning and sequencing of other CTV isolates with different biological properties (an Israeli decline isolate VT and Florida mild isolate T30). During year 1 two major breakthroughs were made which facilitated adjustments in objectives, *i.e.* addition of two new objectives: characterization of the defective RNAs associated with CTV, and investigation of the unexpectedly high molecular diversity of the CTV isolates.

The description of the progress made follows along the lines of these objectives.

III. 1. COMPLETE SEQUENCE OF THE CTV GENOME.

CTV, a definitive member of the closterovirus group has the largest positive-strand RNA genome among all plant viruses, 19,296 nt. Moreover, only one group of animal positive-strand RNA viruses, coronaviruses, has the genome size exceeding that of the CTV genome. The whole process of the cloning and sequencing of the CTV genome was difficult, and it was completed by the mid-year 1 of the project. See the included reprint of the paper which resulted from this portion of the project.

Karasev, A.V., Boyko, V.P., Gowda, S., Nikolaeva, O.V., Hilf, M.E., Koonin, E.V., Niblett, C.L., Cline, K., Gumpf, D.J., Lee, R.F., Garnsey, S.M., Lewandowski, D.J., and Dawson, W.O. 1995. Complete sequence of the citrus tristeza virus RNA genome. *Virology* **208**, 511-520.

The appendix does not contain pages 12-21 inclusive.

III. 2. ASSEMBLY OF THE FULL-LENGTH INFECTIOUS CDNA COPIES OF THE CTV GENOME.

We utilized the known sequence of CTV T36 to resynthesize cDNA clones of specific regions of the CTV genome that could be used to be ligated into a full-genomic cDNA clone engineered to have an SP6 promoter at the 5' terminus and a *NotI* restriction site at the 3' terminus as shown in the figure at right.

RNA transcripts were used to inoculate *N. benthamiana* protoplasts, resulting in replication of CTV RNA (the northern hybridization blot is shown as B in the figure at right). Deletions with the CTV genome also replicate well in protoplasts (shown as C in figure).

III. 3. DEVELOPMENT OF THE PROTOPLAST ASSAY SYSTEM.

To be able to test infectivity of the constructs made we needed a test system to monitor replication of CTV. Since CTV is transmitted mechanically by slash-inoculation with great difficulty only when partially purified virions are used as an inoculum, we turned our attention to the *Nicotiana benthamiana* protoplast system developed for the lettuce infectious yellows virus in B. Falk's laboratory. Indeed, we were able to demonstrate substantial replication of CTV in this *N. benthamiana* system. This replication system accommodated as inoculum purified CTV virions, isolated virus RNA, and eventually full-length CTV RNA transcripts. See included a reprint of the paper which resulted from this sub-project.

Navas-Castillo, J., Albiach-Marti, M.R., Gowda, S., Hilf, M.E., Garnsey, S.M., and Dawson, W.O.

1996. Kinetics of accumulation of citrus tristeza virus RNAs. *Virology* **228**, 92-97.

III. 4. DEFECTIVE RNAS.

During sequencing stage of the project, in the year 1, a significant discovery was made which changed our understanding of the complexity of the CTV problem. Defective RNAs (D-RNAs) were found associated with many CTV isolates, in Israel, United States and elsewhere. It immediately posed additional questions. Are those D-RNAs stable or they constantly being generated *de novo*? What are relations of the D-RNAs with the helper virus, can they suppress replication of the genomic RNA ? How these D-RNAs affect the symptomatology of the CTV infection, are they interfering D-RNAs, or perhaps they intensify disease symptoms ? Since most of these questions directly followed the original research plan we allocated part of the resources, primarily in the Bet Dagan lab, to the research aimed at the D-RNAs. Specifically, the Bet Dagan lab addressed the occurrence of the D-RNAs in different isolates, determined the sequence of several D-RNAs, and analyzed the fine structure of three of them. The Lake Alfred lab assisted in screening of a number of the CTV isolates from Florida and from outside U.S. and Israel. To do this we accessed the comprehensive CTV isolate collection at the USDA facility in Beltsville (MD). This part of the project was closely coordinated with the S.M. Garnsey's lab in the USDA-ARS station in Orlando, FL. See included two papers which resulted from this work on D-RNAs characterization.

Mawassi, M., Karasev, A.V., Mietkiewska, E., Gafny, R., Lee, R.F., Dawson, W.O., and Bar-Joseph,

M. 1995*b*. Defective RNA molecules associated with the citrus tristeza virus. *Virology* **208**, 383-387.

Mawassi, M., Mietkiewska, E., Hilf, M.E., Ashoulin, L., Karasev, A.V., Gafny, R., Lee, R.F.,

Garnsey, S.M., Dawson, W.O., and Bar-Joseph, M. 1995*c*. Multiple species of defective RNAs in plants infected with citrus tristeza virus. *Virology* **214**, 264-268.

III. 5. STRIKING ISOLATE DIVERSITY OF THE CTV ISOLATES.

The second breakthrough was also made during year 1 of the project, and it was also directly related to the sequencing efforts that were underway in Lake Alfred and Bet Dagan. After the first D-RNA, from the Israeli isolate VT, was sequenced it was noted that the two segments of this 2.4-kb RNA composed of the 5' and 3' terminal fragments of the CTV genome, have dramatically different degree of similarity between the isolates VT and the prototype T36 from Florida. Specifically, the VT-derived 5' terminal segment of about 1.1-kb had only 60% identity to the prototype T36, while the VT-derived 3' terminal segment of about 1.3-kb had higher than 90% identity to the T36 prototype. Initially, a recombination between different isolates was suspect, however, completion of the sequence of the Israeli isolate VT confirmed that it was not an inter-isolate recombinant. Instead, a very unusual, asymmetric differences were revealed between the VT and the prototype T36 genomes, when the degree of identity varied from the 40% near the 5' end up to 98% near the 3' end. Two immediate implications were evident. The first one was that the molecular heterogeneity among the CTV isolates may far exceed our expectations, and by some accounts put forward the question are these isolates really different viruses ? The second one was of more practical nature: which part of the virus genome we need to use if we want to differentiate CTV isolates ?

To investigate this phenomenon further, a limited library of cDNA clones was generated on the T3-specific dsRNA template. After hybridization-selection a 600-bp T3-specific clone was isolated, sequenced and compared to the T36 sequence. This clone corresponded to an internal region of the CTV polyprotein gene, between methyltransferase and helicase motifs. To our astonishment, the degree of identity between T3 and T36 sequences did not exceed 68% at the nucleotide level. Surprisingly, the correlation between symptomatology and hybridization strength seemed to be

missing, as the T3-specific probe gave a low level hybridization signal with non-decline isolates, and did not hybridize to a biologically similar T36 isolate. In the prototype T36 genome the same region is represented by the clone k17. Using this k17 region as a label we analyzed sequences of three additional CTV isolates which represented independent hybridization groups for this particular region. These isolates, T3, T30, and VT, are all within 12-13% difference of each other. On the other hand, all three of them are more than 30% different from the T36 prototype. Recently, a California isolate SY568 which produces the most severe symptoms including decline, stem pitting and seedling yellows syndromes, was sequenced in Allan Dodds's lab. Unexpectedly, it appeared to be the closest relative of the Florida mild isolate T30.

Several preliminary conclusions have been inferred from these experiments. 1. CTV in infected plants exists as a complex of diverse genotypes (or isolates), often in mixed infections, with multiple D-RNAs. 2. CTV isolates may have profoundly different genome sequence. 3. The differences are distributed unevenly along the genome. 4. There are at least four sequence groups among CTV isolates which may be distinguished by hybridization. 5. So far there seems to be no immediate correlation between sequences and symptoms.

See included a paper which resulted from this part of the project.

Mawassi, M., Mietkiwska, E., Gofman, R., Yang, G., and Bar-Joseph, M. 1996. Unusual sequence relationships between two isolates of citrus tristeza virus. *J. Gen. Virol.* **77**, 2359-2364.

IV. DESCRIPTION OF COOPERATION.

The level of cooperation between the U.S. and Israeli labs was very high even before the start of this project. However, with the initiation of this project it completely changed and, in fact, went to a new level. The interactions between labs were conducted on a daily basis through mail, fax, and e-mail. Every construct, and every new sequence information was available in both labs practically in real time. These every day contacts led to an explosion of the joint publications driven by several new discoveries which were made exclusively because of this increased level of cooperation. This particularly concerns the discovery of defective CTV RNAs, and the molecular heterogeneity of the CTV isolates. For example the whole concept of D-RNAs was conceived due to e-mail contacts between the Bet Dagan and Lake Alfred labs. For a long time both labs were making major efforts to sequence two different isolates, one from Florida (T36) and another from Israel (VT). For a while sequences were more or less similar, until they reached a point where the VT sequence became completely different from the T36 sequence. What was even more discouraging, the Israeli sequence seemed much shorter. Only when the Florida virus was completely sequenced it became evident that the Bet Dagan lab actually came across a defective RNA composed of relatively small pieces of 5' and 3' termini of the complete virus genome. This discovery which completely re-shaped the research on CTV molecular biology, could not have been made if it were not for contacts between the U.S. and Israeli groups of scientists.

All the technologies created during the project time span have become available to both labs, including the full-length infectious cDNA clone of the CTV, the protoplast assay system, all constructs with different D-RNAs, all artificially produced mutants, let alone all virus-specific primers, and plasmids. Both groups freely shared ideas, technologies, and materials, which

contributed to the success of the project.

Two short trips have been made between Florida and Israel for the 3 year period: in May 1995 M. Bar-Joseph visited Lake Alfred during the Workshop on CTV and Brown Citrus Aphid Problem, and in August 1996 A. Karasev, a postdoctoral researcher working on the project, visited Bet Dagan during the Xth International Congress of Virology held in Jerusalem.

V. EVALUATION OF THE RESEARCH ACHIEVEMENT

The main original objective of the proposal, creation of the genetic system to manipulate the CTV genome, has been accomplished. A full-length, infectious cDNA clone of the Florida decline isolate T36 was built and demonstrated to successfully replicate in protoplasts. This is a major scientific accomplishment in plant virology for the past five years. However, it has not only exceptional scientific impact, but substantial practical implications for a very important plant pathology problem. To fully realize the scale of this achievement, it needs to be reminded where we were at the starting point of the proposal.

By the time of the submission, only about half of the 19,296-nt genome of the Florida decline isolate T36 was sequenced. We have completed the sequence during the first year of this proposal and started building full-length cDNA constructs. This step was found to be more difficult than initially anticipated, as there was no precedent in building the recombinant cDNA construct for a positive-strand RNA virus of this size. Even today our genetic system sets the record for plant and animal RNA viruses alike. We completed building the infectious cDNA copy of the CTV genome during year 3 of the proposal, and optimized the *in vitro* transcription of the full-length 19.3-kb RNA copy. Since there was no assay system available to test our cDNA constructs, during years 1 and 2 we developed our own protoplast system suitable for inoculations with purified CTV, isolated viral RNA, or RNA transcripts. This assay system utilizes *Nicotiana benthamiana* or *Citrus* protoplasts, and allows reliable monitoring of the CTV replication either by immunoblotting or Northern hybridization.

During year 1 of the proposal two significant breakthroughs were made which led to some adjustments in research objectives. First, due to close collaboration between the Lake Alfred and Bet

Dagan labs in their sequencing efforts, defective RNAs were found in an Israeli decline isolate VT. Later they were found in most of the CTV isolates from the U.S., Israel, and elsewhere. To exploit the new possibilities which opened up with this discovery, the Bet Dagan lab concentrated more on this new area, while the Florida labs continued work on the genetic system. Second, in the course of the sequencing efforts the striking genetic diversity between different CTV isolates was revealed in the year 1 of the project. The high level of the diversity between isolates with similar biological symptoms was unexpected and stimulated genome sequencing of several different CTV isolates. However, because of the high level of heterogeneity, it took longer to sequence the genome of the Florida mild isolate T30 than we would anticipate, it was completed during year 3 of the project.

We have created the genetic system for manipulations with the largest plant virus genome on Earth. This system is the basis for the molecular characterization of the CTV pathogenesis, transmission and epidemiology. Numerous CTV-specific clones, primers, or hybrid constructs have been designed and produced. All these tools are available and being used for routine laboratory and diagnostic work both in Florida and in Israel. Different hybrids between decline-inducing isolate T36 and mild isolate T30 are being built or tested for replication in our labs, in order to identify the determinant responsible for the quick decline syndrome. Given the complexity of the CTV infection, and the CTV problem in the field, our initial estimates on reaching the objectives concerning mapping of the disease determinants within the same 3 years now seem too optimistic. We continue work on these objectives which go far beyond the 3 year frame.

VI. CONCLUSIONS.

The main goal of the project, creation of a manipulatable genetic system allowing use of molecular genetic methods to study CTV pathogenesis and biology, has been achieved. Now it is possible to address the whole spectrum of epidemiological problems which could be dealt with before only indirectly. A new class of CTV-specific genetic elements has been discovered, defective RNAs. Using the available genetic system their role in the CTV-induced diseases can be now tested. The striking genetic heterogeneity between CTV isolates has been discovered. And again the genetic system developed can be used to address the role of this heterogeneity in CTV biology. Successful completion of the project led to re-vitalizing the whole field of the CTV molecular biology and genetics, stimulation of interest to the CTV problem from the industry and legislature, regulatory and funding agencies.

VII. LIST OF PUBLICATIONS

- Ashulin, L., Mawassi, M., Yang, G., Frank, A., Gafny, R., Febres, V.J., Niblett, C.L., Lee, R.F., and Bar-Joseph, M. 1996. Native citrus tristeza virus virions are heterodimers consisting of two segments of ~2000 nm and ~85 nm, separately encapsidated by subunits of two different coat proteins. *Phytoparasitica* **24**, 175-176.
- Bar-Joseph, M., Filatov, V., Gofman, R., Yang, G., Hadjinicolis, A., Mawassi, M., Gootwine, E., Weisman, Y., and Malkinson, M. 1997. Booster immunization with a partially purified citrus tristeza virus (CTV) preparation after priming with recombinant CTV coat protein enhances the binding capacity of capture antibodies by ELISA. *J. Virol. Methods* **67**, 19-22.
- Febres, V.J., Ashoulin, L., Mawassi, M., Frank, A., Bar-Joseph, M., Manjunath, K.L., Lee, R.F., and Niblett, C.L. 1996. The p27 protein is present at one end of the citrus tristeza virus particles. *Phytopathology* **86**, 1331-1335.
- Karasev, A.V., Hilf, M.E., Garnsey, S.M., and Dawson, W.O. 1997. Transcriptional strategy of closteroviruses: mapping the 5' termini of the citrus tristeza virus subgenomic RNAs. *J. Virol.* **71**, 6233-6236.
- Karasev, A.V., Nikolaeva, O.V., Mushegian, A.R., Lee, R.F., and Dawson, W.O. 1996. Organization of the 3'-terminal half of beet yellow stunt virus genome and implications for the evolution of closteroviruses. *Virology* **221**, 199-207.
- Karasev, A.V., Boyko, V.P., Gowda, S., Nikolaeva, O.V., Hilf, M.E., Koonin, E.V., Niblett, C.L., Cline, K., Gumpf, D.J., Lee, R.F., Garnsey, S.M., Lewandowski, D.J., and Dawson, W.O. 1995. Complete sequence of the citrus tristeza virus RNA genome. *Virology* **208**, 511-520.
- Mawassi, M., Gafny, R., Gagliardi, D., and Bar-Joseph, M. 1995a. Populations of citrus tristeza virus

contain smaller-than-full-length particles which encapsidate sub-genomic RNA molecules.

J. Gen. Virol. **76**, 651-659.

Mawassi, M., Karasev, A. V., Mietkiewska, E., Gafny, R., Lee, R.F., Dawson, W.O., and Bar-Joseph, M. 1995*b*. Defective RNA molecules associated with the citrus tristeza virus. *Virology* **208**, 383-387.

Mawassi, M., Mietkiewska, E., Hilf, M.E., Ashoulin, L., Karasev, A.V., Gafny, R., Lee, R.F., Garnsey, S.M., Dawson, W.O., and Bar-Joseph, M. 1995*c*. Multiple species of defective RNAs in plants infected with citrus tristeza virus. *Virology* **214**, 264-268.

Mawassi, M., Mietkiewska, E., Gofman, R., Yang, G., and Bar-Joseph, M. 1996. Unusual sequence relationships between two isolates of citrus tristeza virus. *J. Gen. Virol.* **77**, 2359-2364.

Navas-Castillo, J., Albiach-Marti, M.R., Gowda, S., Hilf, M.E., Garnsey, S.M., and Dawson, W.O. 1996. Kinetics of accumulation of citrus tristeza virus RNAs. *Virology* **228**, 92-97.