6

BARD

FINAL REPORT
PROJECT NO. US-874-84

**Maintaining the Use of Sour Orange Rootstock
in the Threat of Citrus Tristeza Virus
by Biological Control**

R. F. Lee, M. Bar-Joseph, R. H. Brlansky, A. Rosner

32

630.72
BAR/LEE
2nd copy

1989

31345

000000115261

a. COVER PAGE

14 July 1989

BARD
P.O. Box 6
Bet Dagan, ISRAEL

BARD Project No. US-874-84

Title of Research Project:

MAINTAINING THE USE OF SOUR ORANGE ROOTSTOCK IN THE THREAT OF CITRUS
TRISTEZA VIRUS BY BIOLOGICAL CONTROL

Names of Investigators:

Principal Investigator: Richard F. Lee

Cooperating investigators: Moshe Bar-Joseph, Ronald H. Brilansky, Arie Rosner

Name and Address of Affiliated Institutions:

Principal Institution: University of Florida, Division of Sponsored
Research, 219 Grinter Hall, Gainesville, FL 32611

Cooperating Institution: The Volcani Center, Agricultural Research
Organization, Bet Dagan, Israel

Project's starting date: October 21, 1985

Type of Report: Final

Signatures:

Richard F. Lee
Principal Investigator
Plant Pathologist

Institution's Authorizing Official
Dillard C. Marshall
Assistant Director of Research

התאחדות המחקר
למזון ולחקר
ב"ח-117

630.72
BAR/LEE : 634.31 : 632.96 : 632.8
2nd copy

b. Table of Contents

a. Cover page	1
b. Table of Contents.	2
c. Abstract	4
d. Scientific Report	5
Introduction	5
Objectives	5
Materials and Methods	5
Selection of CTV strains	5
Purification of virus	6
RNA extractions	6
Isolation and characterization of coat protein	6
Preparation on monoclonal antibodies	6
Serological assays	6
In vitro translation of viral RNA	7
Electrophoresis	7
Results and Discussion	7
CTV situation in Israel and Florida.	7
Distribution of CTV in citrus groves	8
Distribution of CTV in field trees	8
Standardized method to evaluate CTV isolates	9
In situ immunofluorescence detection of CTV	10
Effect of aphid transmission on epidemiology of CTV.	10
Selection of cross protecting CTV isolates	11
Use of serology to differentiate CTV isolates.	12
Isolation of dsRNA	12
Translation of dsRNAs	13

Comparison of relative titer	13
Cytological determinations	13
Selection of cDNA probes for differentiation of CTV.	14
Characterization of CTV coat proteins	14
Cross protection experiments	15
Characterization of the CTV genome	16
Literature cited	17
Figure 1. Restriction site map of CTV T-36 cDNA clones PSDB and P6A6.	20
e. Description of cooperation	21
f. Benefit to agriculture	21
g. List of publications	22

c. Abstract

Mild strain cross protection experiments have been established in Florida and Israel to select mild tristeza strains which can protect against decline induced by severe tristeza strains. In Florida, challenges have been made by using viruliferous aphids, graft challenge, and natural aphid challenge. Greenhouse evaluations are underway to determine if mild CTV strains with cross protecting ability can be quickly selected in a period of a few months. The effect of environment on expression of CTV symptoms and distribution of CTV isolates within trees have been studied. Several methods have been developed to differentiate CTV strains: polypeptide mapping of CTV coat proteins was used to differentiate a decline strain in Spain from nondecline strains, cDNA probes specific for CTV RNA have been selected which are specific for specific strains, a monoclonal antibody has been developed which reacts strongly with severe strains and weakly with mild strains, and enumeration of inclusion bodies. An amplified ELISA assay has been used for sensitive CTV diagnosis. A standardized indexing method has been developed to compare the biological activities of different CTV strains. cDNAs have been prepared from CTV RNA, about 3,000 bases have been sequenced from the 3' end of the CTV genome. In vitro translation of CTV subgenomic dsRNAs has shown that two segments of 0.8 and 1.7 Kbp yield polypeptides in the translates which are immunoprecipitated by antiserum specific for CTV. The research conducted on this project should enable development of better control methods of CTV by enabling the detection of specific severe strains to prevent further propagation in budwood sources and by the selection of mild cross protecting strains of CTV which will reduce tree losses due to CTV.

d. Report

Introduction

Citrus tristeza virus (CTV) is the most economically important citrus virus in the world (Bar-Joseph et al, 1979a, Bar-Joseph et al, 1989). There are numerous CTV strains which vary widely in their biological activity. The destructive aspects of CTV are stem pitting of grapefruit or sweet orange scions regardless of the rootstock, stunting and stem pitting of Mexican lime, slow decline including stunting and dwarfing of plants propagated on sour orange rootstocks, and quick decline of trees on sour orange rootstock. Interest has remained high on maintaining sour orange as a rootstock for citrus because of cold tolerance, high fruit quality, and tolerance to citrus blight, a destructive disease of unknown etiology. Most CTV tolerant rootstocks are very susceptible to citrus blight.

Biological control of severe CTV strains by the use of mild strain cross protection is not a new concept. The phenomenon of cross protection whereby there is an interference in symptom expression by a challenge-inoculated virus on a plant previously infected was first reported by McKinney in 1929. On a practical basis mild CTV strains, selected empirically, are currently used on a large scale in Brazil to protect Pera sweet orange on Rangpur lime rootstock from CTV-induced stem pitting (Costa & Muller, 1980) and also in South Africa to protect against stem pitting on grapefruit (De Lange et al, 1981). Until recently, mild strain cross protection has been tested to a limited extent as a means to protect trees grafted on sour orange rootstocks from CTV-induced decline (Wallace & Drake, 1976). The overall objective of this research was to maintain the use of sour orange rootstock for citrus production by the use of mild strain cross protection of CTV.

The specific objectives of this project were:

1. Determine factors associated with cross-protecting mild CTV strains.
2. This depended on the Development of means with which to differentiate mild and severe strains to determine the events occurring when a mild strain cross protected plant is challenged by a severe strain.
3. Characterization of the CTV genome.

Materials and Methods

Selection of CTV strains. CTV strains used for cross protection tests were selected from field isolations in areas where quick decline was prevalent. The field isolates were aphid transmitted to either Mexican lime or citron plants, then indexed on numerous indicator plants to determine the biological activity of each strain (Garnsey et al, 1987). Strains which indexed as mild were selected for use in cross protection experiments. In both Israel and Florida, a standard collection of CTV

strains were also maintained which represented the range of biological activities found in each respective country. Arrangements were made to use the quarantine facilities of the USDA Beltsville Agricultural Research Center, Maryland, to compare the CTV strains from different areas under common conditions. Side by side evaluation of Florida and Israeli CTV strains in cross protection experiments was also made in South Africa where severe grapefruit stem pitting strains are prevalent.

Purification of virus. Methods used for the purification of CTV include the procedures of Bar-Joseph et al (1985), Lee et al (1987), and Lee et al (1988).

RNA extractions. RNA for use in cDNA cloning was extracted from purified virus preparations using the method of Rosner et al (1983). For use in hybridization, total RNA, dsRNA, or RNA from partially purified virus preparations was extracted using the procedures of Rosner et al (1986). dsRNA extractions were made using the procedure of Lee (1984) or Bar-Joseph et al (1985).

Isolation and characterization of viral coat protein. CTV coat protein was isolated from SDS dissociated virus using the procedure of Lee et al (1988) and Guerri et al (1989). After recovery of CTV coat protein from a preparative SDS polyacrylamide gel, the proteolytic digestions were carried out as described by Lee et al (1988) and the resultant polypeptides separated by electrophoresis on 15% SDS polyacrylamide gels. Amino acid composition of CTV coat proteins were determined on 12, 24, and 72 hour acid hydrolyzed samples using an automated Beckman 120-C amino acid analyzer.

Preparation of monoclonal antibodies. Balb/c mice were initially immunized by intraperitoneal injection with 100 ug purified T-36 CTV in Freund's complete adjuvant. The mice were hyperimmunized by intravenous injections with 50 ug of purified T-36 CTV in 0.2 ml of 0.1 M phosphate buffered saline at 1 and 10 months after the initial immunization. Three days after the final injection spleen cells were harvested and fused with Sp2/0-Ag-14 myeloma cells using the technique of Van Deusen and Whetstone (1981). Two weeks after plating, primary hybridomas were screened by indirect ELISA using plate-trapped antigens (Permar et al, 1989).

Serological Assays. Double antibody sandwich enzyme linked immunosorbent assays (DAS-ELISA) were done using the procedure of Bar-Joseph et al (1979b). Plate-trapped antigen ELISA (PTA-ELISA) were done using the procedure of Permar et al (1989). Modifications were made to DAS-ELISA to develop an ultrasensitive amplified ELISA assay (Dulieu and Bar-Joseph, 1989). Serologically specific electron microscopy assays were done as described by Lee et al (1987). Western blots assays were done as described by Lee et al (1988). Immunoprecipitation of in vitro translation products was done using the procedure of Lee et al (1988) or Dulieu and Bar-Joseph (1989b). Immunospot assays were done as described by Gumpf et al (1985). In situ immunofluorescence for the detection of CTV inclusion bodies was done as described by Brlansky et al (1989).

In vitro translation of virus RNA. Single stranded RNA extracted from purified CTV virions was translated in a rabbit reticulocyte lysate system as described by Lee et al (1988). dsRNAs were collected as described by Dulieu and Bar-Joseph (1989a), denatured with methylmercury, and translated in a reticulocyte lysate in vitro system (Dulieu and Bar-Joseph, 1989b).

Electrophoresis. Viral coat protein and products of in vitro translation were electrophoresed on SDS polyacrylamide gels using the Laemmli system (1970). Double stranded RNA preparations were electrophoresed on non-denaturing polyacrylamide gels using procedures described by Dodds and Bar-Joseph (1983) or Lee (1984). Denaturing agarose gel electrophoresis was performed as described by Rosner et al (1986) and Bar-Joseph et al (1985).

Results and Discussion

Research has progressed on determination of factors associated with cross-protecting mild CTV strains. This work has included biological characterization of severe CTV strains, distribution of mild and severe CTV strains within plants, comparison of titers of mild and severe CTV strains, studies on aphid transmission of mild and severe strains, cytopathic comparison of the effects of mild and severe strains on different hosts. A brief summary of the research results on this objective follows.

The CTV situation in Israel and Florida. A rapidly spreading decline of *Minneola tangelos*, Shamouti and Valencia sweet orange grafted on sour orange rootstock in the Morasha area, in the coastal plain of Israel, was found to be caused by a severe seedling yellows strain of CTV. Repeated ELISA tests revealed great variation in distribution of CTV throughout the canopies, even in declining trees. In a substantial number of the declining trees, samples of up to 10 twigs per tree were not always sufficient for CTV detection. The ELISA value (O.D. 405 nm) in the parts found infected were high, whereas in most of the twigs showing negative ELISA results the virus was absent as indicated by biological indexing. The Morasha strain of CTV also was characterized by rapid annual spread rates. The ratio of declining trees/ELISA-positive trees was proposed as a simple index of strain severity. The epidemiological consequences of the uneven distribution of CTV and the rapid decline were evaluated. Preliminary assays of the Morasha strain of CTV distribution in allemow seedlings following graft inoculation indicate a basopetal spread of the virus.

A survey was conducted in Florida to determine the extent of the CTV epidemic in the state. Extensive tree loss has occurred within a 100 sq. mile area west of Fort Pierce and within a 200 sq. mile area south of LaBelle. Ground and aerial surveys were used to confirm the cause of the decline and the extent of the problem. Tree losses in excess of 50% have occurred in some plantings and have been especially severe during the past few years. Trees declined rapidly and often died within several months. A yellowish-brown discoloration was observed at the

budunion on many, but not all declining trees. Honeycombing in the bark at the budunion was rare. Stunted trees on sour orange also were observed. These apparently originated from propagation of budwood containing a severe stunting isolate of CTV. Results of this work are presented in the appended preprint and reprint:

Ben-Ze'ev, I. S., M. Bar-Joseph, Y. Nitzan, and R. Marcus. 1989. A severe citrus tristeza virus isolate causing the collapse of trees on sour orange before virus is detectable throughout the canopy. *Annals of Applied Biology* 114: in press.

Brlansky, R. H., R. R. Pelosi, S. M. Garnsey, C. O. Youtsey, R. F. Lee, R. K. Yokomi, and R. M. Sonoda. 1986. Tristeza quick decline epidemic in South Florida. *Proc. Fla. State Hort. Soc.* 99: 66-69.

The distribution of CTV in citrus groves in Israel was considered using the data gathered from CTV surveys. A theoretical curve is fitted to the distribution of the number of infected trees in infected plots which contain at least a single infected tree. Recommendations for improving the sampling method for locating infected plots with a low incidence of infection have been presented.

The distribution of CTV within the canopy of field trees was determined by ELISA on individual flushes collected from citrus trees in Florida and South Africa. In Florida, most CTV isolates were unevenly distributed in grapefruit trees, especially in late summer. However, one mild isolate with some cross-protecting ability was consistently distributed throughout the trees regardless of season. CTV was more evenly distributed in sweet orange trees in Florida, but occasionally very young flush tissue was found CTV-free. In South Africa, CTV was found evenly distributed throughout both grapefruit and sweet orange trees. In side by side tests in a greenhouse, South African isolates were highly invasive in recently inoculated plants whereas Florida isolates were not. The even distribution of CTV within trees is a trait which may be important for effective cross protection by mild CTV isolates.

Marsh grapefruit seedlings were inoculated with ten different CTV isolates and planted in two locations in South Africa, one location in the Eastern Transvaal and the other in Natal. The Natal location has a cooler climate from August through January. The purpose of this comparison was to see if all isolates being tested displayed the same biological activity (stem pitting, dwarfing, and severity of symptoms on grapefruit) at each location. Seven of the isolates tested were classified as mild, three as severe. After three years of field evaluation, two of the mild isolates and all three of the severe isolates reacted similarly at both locations. The remaining five mild isolates produced much more severe symptoms at one location as compared to the other location. One mild isolate produced severe stem pitting (rated as severe as the original severe isolates in the test) at the Eastern Transvaal location and very mild pitting at Natal. Another isolate produced severe stem pitting in the Natal location, moderate pitting at the East Transvaal location. There was no correlation

between ELISA titer and severity of CTV isolates, but there were significant differences in virus titer between isolates. The results, being prepared for publication, suggests there is a influence of the environment upon the expression of virus symptoms under field conditions.

Please refer to the attached publications:

Marcus, R., R. Salomon, and M. Bar-Joseph. 1987. The distribution of citrus tristeza virus in citrus groves in Israel. *Journal of Applied Statistics* 14: 15-21.

Lee, R. F., S. M. Garnsey, L. J. Marais, J. N. Moll, and C. O. Youtsey. 1988. Distribution of citrus tristeza virus in grapefruit and sweet orange in Florida and South Africa. pp. 33-38 in: *Proc. 10th Conf. IOCV*, L. W. Timmer, S. M. Garnsey, and L. Navarro, Eds., Univ. of California Press, Riverside.

A standardized method to evaluate the biological properties of CTV isolates was developed. Because there are many isolates of CTV and these isolates vary greatly in their biological activity, it is important to be able to compare the severity of isolates from different locations around the world. The major detrimental biological properties of CTV are decline, stem pitting, and seedling yellows. The standardized method to evaluate the biological properties of CTV incorporates a weighted scoring of the symptoms in five indicator hosts and measurement of the three major disease syndromes. The indicator hosts used are Mexican lime, sour orange, Duncan grapefruit, Madam Vinous sweet orange and a grafted combination of sweet orange on sour orange rootstock. Side by side comparisons of Israel and Florida isolates have been made at the USDA Quarantine Facility, Beltsville Agricultural Research Center, Beltsville, Maryland. Also included in this testing are CTV isolates from most of the major citrus areas of the world. This standardized method of evaluation should be helpful in evaluation of cross protection experiments conducted in different countries. Please refer to attached reprint.

Garnsey, S. M., D. J. Gumpf, C. N. Roistacher, E. L. Civerolo, R. F. Lee, R. K. Yokomi, and M. Bar-Joseph. 1987. Toward a standardized evaluation of the biological properties of citrus tristeza virus. *Phytophylactica* 19: 151-157.

Using the standardized method of evaluation of the biological properties of CTV isolates, the isolates collected from normal appearing and trees undergoing quick decline from several different locations in Florida were evaluated. None of the isolates caused stem pitting on grapefruit or sweet orange nor was seedling yellows detected. Only three of eight locations had CTV isolates which induced quick decline upon indexing. Several CTV isolates collected from quick declining trees in groves with fluctuating water tables did not cause quick decline on the indicator plants and were considered to be relative mild isolates. It seems probable that the stress placed on

the root system of citrus trees on sour orange rootstock in areas with fluctuating water tables may allow some of these relative mild CTV isolates the advantage needed to cause quick decline. Proper water management in Florida Flatwoods locations may reduce tree losses which are now attributed to CTV quick decline. Please refer to the attached preprint.

Lee, R. F. and R. H. Brlansky. 1989. Variation in the biological index of citrus tristeza virus isolates from quick decline affected groves. Proc. Fla. State Hort. Soc. 102: in press.

An in situ immunofluorescence method for the detection of CTV inclusion bodies in plant tissue has been developed. Cryostat-cut thin (30-40 um) section of plants tissue are immersed in TRITC labelled or FITC labelled IgG, rinsed, and viewed with a fluorescence microscope. Orange-red fluorescing bodies were readily observed in phloem fibers, sieve tubes, and parachyma cells of CTV-infected tissue section incubated in TRITC-labelled IgG specific to CTV. Yellow-green fluorescence of the same cells was observed after incubation in FITC-labelled IgG. Autofluorescence was more evident with FITC-labelled IgG than with TRITC-labelled IgG. No fluorescing bodies were observed in sections incubated in normal IgG or in fluorescent labelled IgG specific for either tobacco etch virus or Pierce's disease bacterium or in sections of healthy tissue incubated in TRITC-labelled CTV IgG. The same fluorescing bodies in CTV-infected tissue also were stained by Azure A, indicating that the nucleoprotein-containing inclusion bodies of CTV were the fluorescing structures.

The numbers of inclusion bodies produced by mild and severe CTV isolates in seven citrus hosts were determined. Over all the citrus hosts, the numbers of the inclusion bodies was positively related to strain severity and to virus titer as determined by ELISA. This trend was most evident in the more susceptible hosts such as Mexican lime, Citrus hystrix, Madam Vinous sweet orange, and C. excelsa and less apparent in less susceptible sour orange, rough lemon, and grapefruit. No differences were noted in the types of inclusions produced in the various hosts. Please refer to the attached reprints.

Bransky, R. H. 1987. Inclusion bodies produced in Citrus spp. by citrus tristeza virus. Phytophylactica 19: 211-213.

Bransky, R. H., R. F. Lee, and S. M. Garnsey. 1988. In situ immunofluorescence for the detection of citrus tristeza virus inclusion bodies. Plant Disease 72: 1039-1041.

The effect of aphid transmission on the epidemiology, spread, and control of CTV was studied. The early literature on vector transmission of CTV was reviewed with emphasis on the dramatic increase in transmissibility of CTV. The implications of the change in transmissibility of CTV on spread of severe isolates and the threat of Toxoptera citricida moving into areas where the aphid is not currently present was presented. CTV was successfully transmitted by Aphis gossypii to several Passiflora spp. Two species, P. gracilis and P.

caerulea, retained the virus as detected by ELISA. Successful graft transmission were also made. The virus induced severe leaf chlorosis, stunting and eventual death of P. gracilis plants, and severe stunting of P. caerulea plants and leaves. After transmission from Passiflora spp. back to citrus, there was a loss of one or more of the components of yellow, stunting or stem pitting. Please refer to the attached reprints.

Roistacher, C. N., and M. Bar-Joseph. 1987. Aphid transmission of citrus tristeza virus: a review. *Phytophylactica* 19: 163-167.

Roistacher, C. N., and M. Bar-Joseph. 1987. Transmission of citrus tristeza virus by *Aphis gossypii* and by graft inoculation to and from Passiflora spp. *Phytophylactica* 19: 179-182.

Procedures were developed to evaluate protection of mild CTV isolates against aphid challenge with severe isolates. Mexican lime plants infected with mild Florida CTV isolates were challenged by A. gossypii made viruliferous by feeding on plants infected with severe isolates. After two aphid challenges, the mild isolates were categorized as protective or nonprotective based on evidence of coinfection. This system identified potentially protective isolates and prioritized them for further testing and field trials. Deliberate field vector challenge of sweet orange on sour orange rootstock trees previously inoculated with mild isolates also decreased time needed to evaluate protection of the mild strain. Please refer to attached reprint:

Yokomi, R. K., S. M. Garnsey, R. F. Lee, and M. Cohen. 1987. Use of vectors for screening protecting effects of mild citrus tristeza virus isolates in Florida. *Phytophylactica* 19: 183-185.

Selection of cross protecting CTV isolates and evaluation of temperature effects. Greenhouse experiments have been established using Valencia sweet orange budded onto sour orange rootstock. In a replicated manner, selected mild CTV isolates have been inoculated into the plants with appropriate plants left as healthy control. Some of these plants are being challenged by bud inoculation with a quick decline isolate of CTV while other plants are being challenged by viruliferous aphids carrying the same quick decline isolate. This experiment is being conducted in two temperature regimes, one at 20-25 C, the second at 37-40 C. From this study, we hope to determine if a relative short assay time can be used to determine if a mild CTV isolate can cross protect against CTV quick decline, and what effect temperature has on the ability of mild CTV strains to cross protect.

Progress has been made on objective 2, determination of the events occurring when a mild strain cross protected plants is challenged by a severe strain. To make progress on this objective, several methods and procedures had to be developed.

Several traits have been suggested as important when selecting mild CTV

isolates which may be able to cross protect. The isolate should be mild in all citrus cultivars present in the area, the virus must distribute itself in all parts of the plant and have the ability to invade quickly new growth flushes, potential cross protecting isolates should be evaluated under environmental conditions similar to those in which they will have to perform, and the selected isolates should be aphid vectored. These traits are discussed in the attached reprint:

Lee, R. F., R. H. Bransky, S. M. Garnsey, and R. K. Yokomi.
1987. Traits of citrus tristeza virus important for mild strain cross protection of citrus: the Florida approach. *Phytophylactica* 19: 215-218.

Use of serology assays to differentiate CTV isolates. The sensitivity of the DAS-ELISA has been increased by about 25 fold by the development of an enzyme-amplified ELISA. Using alkaline phosphatase as the enzyme tag, NADP is the primary substrate, which is dephosphorylated to give NAD. The NAD is the catalytic trigger for a redox cycle involving the secondary enzyme/substrate reaction: alcohol dehydrogenase/ethanol, and diaphorase (NADH dye oxidoreductase)/p-iodonitrotetrazolium violet. The use of this enzyme-amplified ELISA allows detection of low concentrations of viruses in plants. Please refer to attached reprint:

Ben-Ze'ev, I. S., A. Frank, and M. Bar-Joseph. 1988. Sensitive detection of two plant viruses by enzyme-amplified ELISA. *Phytoparasitica* 16: 343-349.

A monoclonal antibody (MCA13) has been developed which discriminates CTV isolates. MCA13, produced to the Florida CTV isolates T-36, reacts strongly to decline-inducing, seedling yellows, and stem pitting isolates of CTV. It does not react to mild CTV isolates. Discrimination of CTV isolates was similar in indirect ELISA test with plate-trapped antigen and in DAS-ELISA ELISA tests using a polyclonal antibody coated plates. No reactions occur to extracts of healthy citrus or citrus infected with other viruses. The monoclonal is an IgG2a immunoglobulin. This monoclonal antibody is being used to evaluate several cross protection experiments which have established. Refer to attached preprint:

Permar, T. A., S. M. Garnsey, D. J. Gumpf, and R. F. Lee. 1988. A monoclonal antibody which discriminates isolates of citrus tristeza virus. *Phytopathology* 78: 1559. (Abstract).

Permar, T. A., S. M. Garnsey, D. J. Gumpf, and R. F. Lee. 1989. A monoclonal antibody which discriminates strains of citrus tristeza virus. *Phytopathology* 79: in press.

Isolation of dsRNA. A simple method was developed to isolate dsRNA segments from disulfide crosslinked polyacrylamide gels. After visualization over an UV light, the dsRNA bands were excised from the gel and dissolved using 2-mercaptoethanol. The dsRNA was then purified from the solubilized fractions by specific adsorption on microgranular cellulose and eluted with a small volume of water. Refer to attached preprint.

Dulieu, P. and M. Bar-Joseph. 1989. Rapid isolation of double stranded RNA segments from disulfide crosslinked polyacrylamide gels. *Journal of Virol. Methods*: in press.

Translation of CTV dsRNAs. Several dsRNA segments have been isolated from CTV infected plants and translated, after denaturation, in a reticulocyte lysate cell free system. Only two dsRNA segments of 0.8 and 1.7 Kbp were efficiently translated and showed a consistent pattern of translation products. The major translation products of the 0.8 Kbp dsRNA and the 1.7 Kbp dsRNA were a 27 Kd polypeptide and a 21 Kd polypeptide, respectively, which were immunoprecipitated by antisera prepared against CTV coat protein. The evidence suggests that the CTV coat protein is translated from a subgenomic viral messenger RNA. Please refer to the attached preprint.

Dulieu, P. and M. Bar-Joseph. 1989. In vitro translation of the citrus tristeza virus coat protein from an 0.8 Kbp double stranded RNA segment. *J. Gen. Virol.* :in press.

Comparison of relative titers by infectivity and hybridization. Infectious RNAs were extracted from viroid infected citrus. Portions of the RNAs were radioactive labelled using kinase and used as self probes in hybridization assays where the samples were spotted on membranes in 10 fold dilutions. Other portions were used for dilution endpoint infectivity assays. There was a very high correlation between the highest dilution on the membranes which hybridized with the self labelled probe and the infectivity index determined by dilution endpoint infectivity assays. These methods may be useful with other viruses including CTV. Refer to attached abstracts:

Garnsey, S. M., R. F. Lee, and J. S. Semancik. 1988. Lack of cross protection between citrus exocortis viroid and citrus viroids associated with mild symptoms in etrog citron. pp. 69 in *Proceedings of the 5th International Congress of Plant Pathology*, Kyoto, Japan, August 1988. (Abstract).

Lee, R. F. and S. M. Garnsey. 1987. Comparison of relative titer of mild and severe citrus viroids by infectivity and hybridization. *Phytopathology* 77: 1743 (Abstract).

Cytological determination of the effect of various CTV isolates on the bud union of sweet orange on sour orange rootstock. Bud union samples of greenhouse grown sweet orange on sour orange rootstock plants infected with various Florida mild and severe isolates of CTV were excised and cryosectioned. Sections were stained using thioin, a thiazine strain, prior to viewing under the light microscope. Previous studies have shown the usefulness of thionin in staining necrotic plant tissue. The intensity of stain in the phloem tissue in 8-10 sections per sample was rated on a 0 to 3 scale with 3 indicating intense heavy staining. Staining reactions were compared to the biological index rating for each virus isolate. Other experiments to confirm these results have now been established. Please refer to the attached abstract:

Brlansky, R. H., and S. M. Garnsey. 1988. Cytological determination of the effect of various citrus tristeza virus isolates on the budunion of sweet orange on sour orange rootstock. pp. 422 in: Proc. 10th Conf. IOCV, L. W. Timmer, S. M. Garnsey, and L. Navarro, Eds., Univ. of California Press, Riverside. (Abstract).

Selection of cDNA probes capable of differentiating CTV isolates. cDNA containing plasmids (refer to next section on characterization of CTV genome) have been selected from libraries made against mild and severe CTV isolates which are capable to recognizing and hybridizing to RNAs extracted from selected mild or severe isolates and do not hybridize with other isolates. These cDNA probes capable of differential hybridization are currently being used to evaluate the relative replication rates of the selected CTV isolates in cross protection tests.

Characterization of CTV coat proteins and their use to differentiate CTV isolates. The coat proteins of five Florida CTV isolates were characterized by polyacrylamide gel electrophoresis, Western blotting followed by serological probing, and polypeptide mapping on SDS polyacrylamide gels following treatment with proteolytic enzymes. All Florida isolates had a major coat protein (CP1) of about Mr 23,000 and a minor coat protein (CP2) of about Mr 21,000, both of which reacted with CTV-specific antisera when using Western blot and ELISA assays. The amino acid composition of CP1 was similar to that reported for the coat proteins of other closteroviruses. Polypeptide maps of CP1 and CP2 were similar. In vitro translation of RNA extracted from purified CTV virions produced proteins of various sizes, but only one peptide of about Mr 26,000 was immunoprecipitated with CTV-specific antiserum. When the polypeptide maps of the Florida CTV isolates were compared, similar patterns were obtained for all isolates with only minor difference between some isolates. Please refer to the attached reprint.

Lee, R. F., L. A. Calvert, J. Nagel, and J. D. Hubbard. 1988. Citrus tristeza virus: characterization of coat proteins. Phytopathology 78: 1221-1226.

When the polypeptide mapping was applied to exotic CTV isolates present in the CTV collection at the USDA Quarantine Facility, Beltsville Agriculture Research Center, Beltsville, MD, differentiation of CTV isolates could be made between isolates having similar biological properties and the same double-stranded RNA pattern. Differentiation could be made of the CTV isolate introduced into Spain which causes decline of trees on sour orange rootstock from the isolates already present in the country. Please refer to the attached reprint and preprint.

Lee, R. F., and L. A. Calvert. 1987. Polypeptide mapping of citrus tristeza virus strains. Phytophylactica 19: 205-210.

Guerri, J., P. Moreno, and R. F. Lee. 1989. Identification of citrus tristeza virus strains by peptide maps of virion coat protein. *Phytopathology* 79: in press.

Cross Protection Experiments. In Israel two commercial citrus groves, in which heavy infection with CTV has been observed during the last three years and where CTV eradication has been discontinued, were selected for cross protection experiments. The two plots, one located at Morasha and the second near Hedera were tested by ELISA to locate previously infected trees and visually inspected to locate collapsing trees. At both locations the dominate CTV isolates cause severe seedling yellows reaction, and a large proportion of the trees were found to collapse before the virus was systemically spread throughout the tree canopy as detected by ELISA.. The mild CTV isolates selected for cross protection originated from field trees on sensitive combinations known to carry the disease for several years without showing adverse effects of the tristeza disease. Glasshouse tests indicated that these mild isolates reach high ELISA titers in the sour orange rootstock. Eight rows of Minneola tangelo trees (c 160 trees) at Morasha and 23 rows of Valencia trees at Hedera (c 400 trees) were chip budded on a suitable branch at a height of 50-120 cm, during May 1987. Tests were carried out to assess protective strain distribution following inoculation and to evaluate the protective efficiency following challenge inoculation with the dominant severe CTV isolate prevalent in each location. A third site for cross protection is currently under evaluation. Unlike the two previously selected locations, the new experimental plot was selected because the number of infected trees is still low (5%) and as a result there is little danger of losing a large proportion of experimental trees before the protective agent will be established even at an $r > 3$.

In Florida the field test (located at two locations) for the empirical evaluation of 15 mild CTV isolates for cross protection potential has been maintained. This test was challenged with local severe CTV strains using *Aphis gossypii* as vectors. The challenge was made in March 1987. Data has been collected in November 1986, October 1987 and November 1988 on tree growth and CTV titer in each tree was estimated by quantitative ELISA on tissue collected at the same physiological age.

The experiment in the Southwest Flatwoods location in Florida where mature pineapple trees on sour orange rootstock were inoculated with two mild CTV isolates in an block where quick decline CTV was rampant came to an abrupt end when the grower-cooperator pulled the trees after two years. At the last evaluation, one of the mild strains appeared to slow the decline of trees whereas the other mild strain and the controls which had not been inoculated with mild strains were declining rapidly. The dsRNA profiles indicated the one mild strain had been distributed throughout the trees into which it had been inoculated inspite of a previous CTV infection in the tree.

A field experiment has been established in Florida to evaluate 13 mild CTV strains in Hamlin on sour orange and 11 mild strains in red

grapefruit on sour orange. These trees were graft challenged on 9 Oct 87 with the infected buds being removed after six weeks, a second challenge was made in March 1988 with the buds being removed after seven weeks. These trees are being monitored by growth measurements, quantitative ELISA, and with MCA13 monoclonal antibody to detect the severe CTV challenge strain.

Sour orange type rootstocks, Gou Tou Cheng and Zhu Laun, have been obtained from China, grown out, budded with commercial orange varieties, and being evaluated for tolerance to CTV in greenhouse and field experiments in both Florida and Israel. These experiments have just been initiated and will need to be evaluated for several years to determine CTV tolerance and horticultural performance.

Progress made on objective 3: characterization of the CTV genome.

A library of cDNA clones were made using citrus variegation virus (CVV) RNA as a model system to develop the procedure of polyadenylating the viral RNA, then making a cDNA:RNA hybrid nucleic acid which is then inserted into the Puc 19 plasmid vector. Over 40 clones from this library were confirmed to be viral specific by hybridization analysis and were sized by restriction digest analyses. One clone, approximately 1750 bases, was complementary to RNA 3 and RNA 4 of the CVV genome by Northern blot analysis. Another clone of over 800 bases was shown to be complementary to the RNA 2 of CVV. This clone also hybridized with a fifth species of RNA which suggested that the RNA 4a band is a subgenomic product of RNA 2. These clones were used as probes to characterize the homology between two isolates of CVV and one isolate of citrus leaf rugose virus. A summary of this work has been published, please refer to the attached reprint:

Calvert, L. A., R. F. Lee, and E. Hiebert. 1988.

Characterization of the RNA species of citrus variegation virus with complementary DNA clones. pp. 327-342 in: Proc. 10th Conf. IOCV, Univ. of California, Riverside.

In Israel a new CTV library has been constructed in a Bluescript plasmid vector. Since our recent in vitro translation results indicated the absence of a poly A tail at the 3' end of the CTV genome, we added a poly A to the viral RNA and cDNA synthesis was carried out by the common methods. The ends of the DNA were ligated to Bam H1 linkers and the constructs were introduced into the Bam H1 site of the Blue script vector. The DNA was used to transform JM101 cells and colonies surviving on amp are currently being evaluated.

Complementary DNA libraries have been prepared to the mild Florida T-30 CTV strain and the Florida severe seedling yellows T-36 strain using the cDNA:RNA hybrid procedure and Puc 19 vector as previously described. A clone from the T-30 library, designated 30F6, was partially sequenced and an open reading frame was identified. There were similarities between the amino acid composition of the CTV capsid protein and the predicted protein encoded by the clone 30F6. Two clones from the T-36 cDNA library, designated P6A6 and P5B8 (7350 and 8500 bases in length, respectively), shared homology for approximately

2200 bases and represented nearly 70% of the CTV genome (Figure 1). The 5' and 3' orientation of the clones as compared with the CTV-RNA was determined. Portions of the clone P6A6, which were proximal to the 3' end of the CTV RNA, were sequenced. An open reading frame coding for at least 109 amino acids and terminating with a TAA stop codon was resolved. Within another subclone of P6A6, a sequence consisting of 13A, 1 G, and 7 A bases was found. This area may be similar to the intergenic region of brome mosaic virus RNA 3 that contains a tract of 16-22 adenine bases.

A 5000 base CTV-cDNA fragment was subcloned into open reading frame expression vectors (pORF-1 and pORF-2) and transformed into *Escherichia coli* strains MH-3000 and TK-1046. The resulting blue colonies were selected and confirmed to contain CTV-cDNA inserts by restriction and hybridization analyses. Four distinct trihybrid fusion proteins were identified by use of SDS polyacrylamide gels, blotting to nitrocellulose, and western blotting using antisera specific for B-galactosidase. One fusion protein, designated O2A7 and produced by a 100 base clone, was eluted from SDS polyacrylamide gels and used as an immunogen. The antiserum obtained reacted with several *E. coli* proteins as well as B-galactosidase, but did not reach with any protein which appeared to be related to CTV or present in CTV-infected tissue. Limited portions of the clone designated P6A6, which putatively represents 3' proximal terminus, have been sequenced in order to identify additional restriction sites and the extent of open reading frames. Please refer to the attached abstracts, a manuscript is being prepared.

Calvert, L. A., R. F. Lee, and E. Hiebert. 1986. Complementary DNA cloning of citrus tristeza virus and the expression of viral proteins in *E. coli*. *Phytopathology* 76: 1090 (Abstract).

Calvert, L. A., R. F. Lee, and E. Hiebert. 1987. Citrus tristeza virus partial molecular characterization with complementary DNA clones. *Phytopathology* 77: 1743 (Abstract).

Literature cited

Bar-Joseph, M., S. M. Garnsey, D. Gonsalves. 1979. The closteroviruses: a distinct group of elongated plant viruses. *Advances in Virus Res.* 25: 93-168.

Bar-Joseph, M., S. M. Garnsey, D. Gonsalves, M. Moscovitz, D. E. Purcifull, M. F. Clark, and G. Loebenstein. 1979. The use of enzyme linked immunosorbent assay for detection of citrus tristeza virus. *Phytopathology* 69: 190-194.

Bar-Joseph, M., D. J. Gumpf, J. A. Dodds, A. Rosner, and I. Ginzburg. 1985. A simple purification method for citrus tristeza virus and estimation of its genome size. *Phytopathology* 75: 195-198.

Bar-Joseph, M., R. Marcus, and R. F. Lee. 1989. The continuous challenge of citrus tristeza virus control. *Annual Rev. Phytopathology* 27: in press.

- Brlansky, R. H. 1987. Inclusion bodies produced in Citrus spp. by citrus tristeza virus. *Phytophylactica* 19: 211-213.
- Brlansky, R. H., R. F. Lee, and S. M. Garnsey. 1988. In situ immunofluorescence for the detection of citrus tristeza virus inclusion bodies. *Plant Disease* 72: 1039-1041.
- Costa, A. S., and G. W. Muller. 1980. Tristeza control by cross protection: a U.S.-Brazil cooperative success. *Plant Disease* 64: 538-541.
- De Lange, J. H., S. P. Van Vuuren, and G. S. Bredell. 1981. Groeipunt-entende suiker sitrusklone vir die superplantskema van virusse. *Subtropica* 2(5): 11-16.
- Dodds, J. A. and M. Bar-Joseph. 1983. Double-stranded RNA from plants infected with closteroviruses. *Phytopathology* 73: 419-423.
- Dulieu, P. and M. Bar-Joseph. 1989. Rapid isolation of double stranded RNA segments from disulfide crosslinked polyacrylamide gels. *Journal of Virol. Methods*: 77-84.
- Dulieu, P. and M. Bar-Joseph. 1989. In vitro translation of the citrus tristeza virus coat protein from an 0.8 Kbp double stranded RNA segment. *J. Gen. Virology* : in press.
- Garnsey, S. M., D. J. Gumpf, C. N. Roistacher, E. L. Civerolo, R. F. Lee, R. K. Yokomi, and M. Bar-Joseph. 1987. Toward a standardized evaluation of the biological properties of citrus tristeza virus. *Phytophylactica* 19, 151-157.
- Guerri, J., P. Moreno, and R. F. Lee. 1989. Identification of citrus tristeza virus strains by peptide maps of virion coat protein. *Phytopathology* 79: in press.
- Gumpf, D. J., G. Zheng, P. Moreno, and J. M. Diaz. 1987. Production and evaluation of specific monoclonal antibodies to citrus tristeza virus strains. *Phytophylactica* 19: 159-161.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* 227: 680-685.
- Lee, R. F. 1984. Use of double stranded RNAs to diagnose citrus tristeza virus strains. *Proc. Fla. State Hort. Soc.* 97: 53-56.
- Lee, R. F., L. A. Calvert, J. Nagel, and J. D. Hubbard. 1988. Citrus tristeza virus: characterization of coat proteins. *Phytopathology* 78: 1221-1226.
- Lee, R. F., S. M. Garnsey, R. H. Brlansky, and A. C. Goheen. 1987. A purification procedure for enhancement of citrus tristeza virus yields and its application to other phloem-limited viruses.

Phytopathology 77: 543-549.

McKinney, H. H. 1929. Mosaic diseases in the Canary Islands, West Africa, and Gibraltar. J. Agric. Res. 39: 557-578.

Permar, T. A., S. M. Garnsey, D. J. Gumpf, and R. F. Lee. 1989. A monoclonal antibody which discriminates strains of citrus tristeza virus. Phytopathology 79: in press.

Rosner, A., I. Ginzburg, and M. Bar-Joseph. 1983. Molecular cloning of complementary DNA sequences of citrus tristeza virus RNA. J. Gen. Virol. 64: 1757-1763.

Rosner, A., R. F. Lee, and M. Bar-Joseph. 1986. Differential hybridization with cloned cDNA sequences for detecting a specific isolate of citrus tristeza virus. Phytopathology 76: 820-826.

Van Duesen, R. A., and C. A. Whetstone. 1981. Practical aspects of producing and using antiviral monoclonal antibodies as diagnostic reagents. Proc. 24th Ann. Mtg. Amer. Assn. Vet. Lab. Diagnosticians 24: 211-228.

Wallace, J. M. and R. J. Drake. 1976. Progress report of studies in California on pre-immunization against tristeza in budded citrus trees. Pages 58-62 in: Proc. 7th Conf. Intern. Organ. Citrus Virol., E. C. Calavan, ed. IOCV, Riverside.

Figure 1. Restriction site map of the CTV T-36 cDNA clones P5D8 and P6A6. The position of the restriction sites mapped to the clones are designated using the 5' end as base 1 and the 3' end as 13,600. The Hind III sites (H) are at bases 400 and 4500, the Sph I sites (F) are at base 3100 and 7650, the Sma I sites (R) are at bases 3500, 8200, 9100, 11,300, and 12,800 and 13,100, the Eco RI sites (E) are at bases 4300, 7700, 10,400, and 10,750, the Xba I site (X) is at base 4600, the Sal I sites (Q) are at bases 6,300 and 7,000, the Sst I sites (S) are at bases 7250, 8000, and 11,400, the Pst I site (P) is at base 11,200, the Bam HI site (B) is at 11,850, and the Kpn I sites are at bases 12,300 and 12,750. The Sma I site at base 8,200 is unique to the P5D8 clone. All positions are approximate and there are additional restriction sites for Hind III, Sph I, Kpn I, and Eco RI whose positions have not been unambiguously mapped. These clones are in the vector pUC-19 and the Hind III site in the polylinker region is at the 5' termini of both clones. They are inserted at the Pst I site.

d. Description of cooperation

The degree of cooperation is, without question, the reason for the progress and the success of this project. The use of electronic mail has had the effect of putting our laboratories side by side. It has permitted constant contact, rapid exchange of ideas, questions, and data. This close communication has been augmented by several visits: all participants were together to plan the research done for this project while in South Africa in Nov. 1985; in Nov. 1986 we were able to meet again in Spain to compare results obtained and coordinate future research activities; in Fall, 1988, the U.S. investigators visited the laboratory facilities of investigators in Israel and exchange research results; in Spring, 1989, Dr. Bar-Joseph visited the laboratories in Florida for comparison of research results and work on manuscripts.

Two locations have been utilized which permit side by side comparisons of U.S. and Israeli CTV isolates. Greenhouse comparisons may be conducted at the USDA Plant Quarantine Facility, Beltsville, Maryland, and field evaluations are being conducted at the Citrus and Subtropical Fruit Research Institute, Nelspruit, South Africa. While some aspects of this project have been done in specific locations because of expertise and equipment, these locations have allowed more immediate utilization of each others results.

A feeling of cooperation and commitment to controlling citrus tristeza virus has resulted as a result of this project and the close communication it has fostered between these investigators. The spirit of cooperation is evident upon examination of the publications which have resulted from this project.

f. Benefit to agriculture.

Citrus tristeza virus is the most important virus disease of citrus in the world. In many areas where severe CTV isolates are now present, mild strain cross protection is a viable control strategy. There are still many areas of the world where citrus is grown on sour orange rootstock. As severe CTV isolates encroach on these areas, timely implementation of mild strain cross protection to prevent decline and loss of these citrus industries would be a viable control strategy. Because most citrus areas have abandoned sour orange and planted CTV tolerant rootstocks, little was known about the feasibility of mild strain cross protection to protect against CTV-induced decline on sour orange. The results of this project has done a lot to change this.

As a result of this project, methods are available to ensure that new plantings in the field are not harboring severe CTV. A standardized method has been developed so all major biological activities of a specific CTV isolate can be determined and compared to other isolates from other parts of the world. Progress has been made on rapid methods to identify selected severe CTV isolates: a monoclonal antibody has been developed and selected which recognizes most severe isolates and does not recognize mild isolates, cDNAs have been selected which are

specific for selected severe isolates, the use of proteolytic digestion of viral coat protein followed by polypeptide mapping on SDS-PAGE gels was able to detect the introduced decline causing CTV isolate in Spain from the endemic CTV isolates, the enumeration of inclusion bodies produced in certain hosts can indicate the severity of the CTV isolate when appropriate mild and severe CTV isolates are included as standards, selected CTV isolates can often be identified by the profile of dsRNAs on PAGE gels.

More is known about the epidemiology of CTV and the factors which need to be considered when implementing mild strain cross protection. As a result of the epidemiology studies, better sampling methods have been developed for locating infected plots in areas having low infection rates.

Information on the factors which effect the efficiency of cross protection in the field has been obtained. This includes the distribution of CTV within the tree canopy, relative titer of CTV in field trees, effect of environmental conditions, effect of factors such as fluctuating water tables on predisposing trees to CTV decline, and cytopathic effect on budunion dysfunction. Progress is being made on a more rapid method to prescreen mild CTV isolates to determine their cross protecting ability. With the development of greenhouse methods to evaluate cross protecting potential in one year and knowledge of traits which seem to associate with cross protecting isolates, the selection and screening of mild isolates for cross protecting ability has been greatly speeded up and the evaluation is less empirical.

Basic knowledge of the CTV genome has been learned. We know the genome lacks the 3' poly A tail found in many plant viruses. The single stranded RNA genome serves as a plus sense messenger RNA. Several libraries have been prepared against mild and severe CTV isolates. These libraries are in vectors which will facilitate future sequencing and characterization of the CTV genome. Restriction maps have been prepared for some of the larger clones, subclones have been sequenced, and, in some instances, inserted in expression vectors and trihybrid proteins produced. The coat protein gene for CTV has not been identified. The libraries of CTV clones knowledge gained will aid greatly in further characterization of CTV.

g. List of publications

- Bransky, R. H., R. R. Pelosi, S. M. Garnsey, C. D. Youtsey, R. F. Lee, R. K. Yokomi, and R. M. Sonoda. 1986. Tristeza quick decline epidemic in South Florida. Proc. Fla. State Hort. Soc. 99: 66-69.
- Calvert, L. A., R. F. Lee, and E. Hiebert. 1986. Complementary DNA cloning of citrus tristeza virus and the expression of viral proteins in E. coli. Phytopathology 76: 1090 (Abstract).
- Lee, R. F., R. H. Bransky, S. M. Garnsey, and R. K. Yokomi. 1987. Traits of citrus tristeza virus important for mild strain cross protection of citrus: the Florida approach. Phytophylactica 19:

215-218.

- Lee, R. F., and L. A. Calvert. 1987. Polypeptide mapping of citrus tristeza virus strains. *Phytophylactica* 19: 205-210.
- Yokomi, R. K., S. M. Garnsey, R. F. Lee, and M. Cohen. 1987. Use of vectors for screening protecting effects of mild citrus tristeza virus isolates in Florida. *Phytophylactica* 19: 183-185.
- Brlansky, R. H. 1987. Inclusion bodies produced in Citrus spp. by citrus tristeza virus. *Phytophylactica* 19: 211-213.
- Garnsey, S. M., D. J. Gumpf, C. N. Roistacher, E. L. Civerolo, R. F. Lee, R. K. Yokomi, and M. Bar-Joseph. 1987. Toward a standardized evaluation of the biological properties of citrus tristeza virus. *Phytophylactica* 19: 151-157.
- Roistacher, C. N., and M. Bar-Joseph. 1987. Aphid transmission of citrus tristeza virus: a review. *Phytophylactica* 19: 163-167.
- Roistacher, C. N., and M. Bar-Joseph. 1987. Transmission of citrus tristeza virus by *Aphis gossypii* and by graft inoculation to and from *Passiflora* spp. *Phytophylactica* 19: 179-182.
- Lee, R. F., S. M. Garnsey, R. H. Brlansky, and A. C. Goheen. 1987. A purification procedure for enhancement of citrus tristeza virus yields and its application to other phloem-limited viruses. *Phytopathology* 77: 543-549.
- Marcus, R., R. Salomon, and M. Bar-Joseph. 1987. The distribution of citrus tristeza virus in citrus groves in Israel. *Journal of Applied Statistics* 14: 15-21.
- Lee, R. F. and S. M. Garnsey. 1987. Comparison of relative titers of mild and severe citrus viroids by infectivity and hybridization. *Phytopathology* 77: 1743 (Abstract).
- Calvert, L. A., R. F. Lee, and E. Hiebert. 1987. Citrus tristeza virus partial molecular characterization with complementary DNA clones. *Phytopathology* 77: 1743 (Abstract).
- Lee, R. F., S. M. Garnsey, L. J. Marais, J. N. Moll, and C. O. Youtsey. 1988. Distribution of citrus tristeza virus in grapefruit and sweet orange in Florida and South Africa. pp. 33-38 in: *Proc. 10th Conf. IOCV*, L. W. Timmer, S. M. Garnsey, and L. Navarro, Eds., Univ. of California Press, Riverside.
- Calvert, L. A., R. F. Lee, and E. Hiebert. 1988. Characterization of the RNA species of citrus variegation virus with complementary DNA clones. pp. 327-342 in: *Proc. 10th Conf. IOCV*, Univ. of California, Riverside.
- Brlansky, R. H., R. F. Lee, and S. M. Garnsey. 1988. In situ immunofluorescence for the detection of citrus tristeza virus

inclusion bodies. Plant Disease 72: 1039-1041.

Lee, R. F., L. A. Calvert, J. Nagel, and J. D. Hubbard. 1988. Citrus tristeza virus: characterization of coat proteins. Phytopathology 78: 1221-1226.

Brlansky, R. H., and S. M. Garnsey. 1988. Cytological determination of the effect of various citrus tristeza virus isolates on the budunion of sweet orange on sour orange rootstock. pp. 422 in: Proc. 10th Conf. IOCV, L. W. Timmer, S. M. Garnsey, and L. Navarro, Eds., Univ. of California Press, Riverside. (Abstract).

Garnsey, S. M., R. F. Lee, and J. S. Semancik. 1988. Lack of cross protection between citrus exocortis viroid and citrus viroids associated with mild symptoms in etrog citron. pp. 69 in Proceedings of the 5th International Congress of Plant Pathology, Kyoto, Japan, August 1988. (Abstract).

Brlansky, R. H., D. S. Howd, C. L. Davis, and R. F. Lee. 1988. Differences in the number of inclusion bodies produced by mild and severe citrus tristeza virus strains in four citrus hosts. Phytopathology 78: 1524. (Abstract).

Permar, T. A., S. M. Garnsey, D. J. Gumpf, and R. F. Lee. 1988. A monoclonal antibody which discriminates isolates of citrus tristeza virus. Phytopathology 78: 1559. (Abstract).

Garnsey, S. M. and R. F. Lee. 1988. Citrus tristeza virus. pp. 48-50 in: Compendium of Citrus Diseases, Whiteside, Timmer, and Garnsey, Eds., APS Press, St. Paul, Minn.

Ben-Ze'ev, I. S., A. Frank, and M. Bar-Joseph. 1988. Sensitive detection of two plant viruses by enzyme-amplified ELISA. Phytoparasitica 16: 343-349.

Bar-Joseph, M., R. Marcus, and R. F. Lee. 1989. The continuous challenge of citrus tristeza virus control. Annual Review of Phytopathology 27:

Ben-Ze'ev, I. S., M. Bar-Joseph, Y. Nitzan, and R. Marcus. 1989. A severe citrus tristeza virus isolate causing the collapse of trees on sour orange before virus is detectable throughout the canopy. Ann. Appl. Biol. 114: in press.

Permar, T. A., S. M. Garnsey, D. J. Gumpf, and R. F. Lee. 1989. A monoclonal antibody which discriminates strains of citrus tristeza virus. Phytopathology 79: in press.

Guerri, J., P. Moreno, and R. F. Lee. 1989. Identification of citrus tristeza virus strains by peptide maps of virion coat protein. Phytopathology 79: in press.

Lee, R. F. and R. H. Brlansky. 1989. Variation in the biological index of citrus tristeza virus isolates from quick decline

affected groves. Proc. Fla. State Hort. Soc. 102: in press.

Dulieu, P. and M. Bar-Joseph. 1989. Rapid isolation of double stranded RNA segments from disulfide crosslinked polyacrylamide gels. Journal of Virol. Methods: 77-84.

Bar-Joseph, M., R. Marcus, and R. F. Lee. 1989. The continuous challenge of citrus tristeza virus control. Annual Review of Phytopathology 27: in press.

Dulieu, P. and M. Bar-Joseph. 1989. In vitro translation of the citrus tristeza virus coat protein from an 0.8 Kbp double stranded RNA segment. J. Gen. Virology : in press.

RECEIVED

NOV

1989