**BARD Final Scientific Report
Cover Page**

Date of Submission of the report:

BARD Project Number: US-3171-99C

Project Title: Creating an ally from an adversary: genetic manipulation of Citrus tristeza virus.

Investigators

Principal Investigator (PI): William O. Dawson

Co-Principal Investigator (Co-PI): Moshe Bar-Joseph

Collaborating Investigators:

Institutions

University of Florida

ARO, The Volcani Center

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Abbreviations commonly used in the report, in alphabetical order:

Budget: IS: \$150,000

US: \$140,000

Total: \$290,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

BARD Final Scientific Report Cover Page

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	10	2	1	13
Submitted, in review, in preparation				
Invited review papers				
Book chapters	2	0	2	4
Books				
Master theses				
Ph.D. theses			2	2
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Munir Mawassi

Tatanini Satyanarayana

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	0	3		3
Longer Visits (Sabbaticals)	0	1		1

Description of Cooperation:

Although we meet often through visits, the major component of our interaction results from daily E-mails, which we have utilized as an effective means of communication and cooperation.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

ABSTRACT

TITLE: Creating an Ally from an Adversary: Genetic Manipulation of Citrus Tristeza Virus.

PIs: William O. Dawson and Moshe Bar-Joseph

OBJECTIVES:

- 1) Engineer CTV to protect trees from diseases caused by severe isolates of CTV.**
- 2) Begin designing virus-based vectors to transiently express foreign sequences in citrus trees.**

Citrus is one of the major agricultural crops common to Israel and the United States, important in terms of nutrition, foreign exchange, and employment. The economy of both citrus industries have been chronically plagued by diseases caused by *Citrus tristeza virus* (CTV). The short term solution until virus-resistant plants can be used is the use of mild strain cross-protection. We are custom designing “ideal” protecting viruses to immunize trees against severe isolates of CTV by purposely inoculating existing endangered trees and new plantings to be propagated as infected (protected) citrus budwood. We crossed the substantial technological hurdles necessary to accomplish this task which included developing an infectious cDNA clone which allows *in vitro* manipulation of the virus and methods to then infect citrus plants. We created a series of hybrids between decline-inducing and mild CTV strains, tested them in protoplasts, and are amplifying them to inoculate citrus trees for evaluation and mapping of disease determinants.

We also extended this developed technology to begin engineering transient expression vectors based on CTV as tools for genetic improvement of tree crops, in this case citrus. Because of the long periods between genetic transformation and the ultimate assay of mature tree characteristics, there is a great need for an effective system that allows the expression or suppression of target genes in fruiting plants. Virus-based vectors will greatly expedite progress in citrus genetic improvement. We characterized several components of the virus that provides necessary information for designing virus-based vectors. We characterized the requirements of the 3'-nontranslated replication promoter and two 3'-ORF subgenomic (sg) mRNA controller elements. We discovered a novel type of 5'-terminal sgRNAs and characterized the *cis*-acting control element that also functions as a strong promoter of a 3'-sgRNA. We showed that the p23 gene controls negative-stranded RNA synthesis and expression of 3' genes. We identified which genes are required for infection of plants, which are host range determinants, and which are not needed for plant infection. We continued the characterization of native dRNA populations and showed the presence of five different classes including class III dRNAs that consists of infectious and self-replicating molecules and class V dRNAs that contain all of the 3' ORFs, along with class IV dRNAs that retain non-contiguous internal sequences. We have constructed and tested in protoplasts a series of expression vectors that will be described in this proposal.

ACHIEVEMENTS

We believe that we are near developing methods to manage the diseases for growers in Israel and the US. We are approaching the engineering of “ideal” viruses that will protect citrus trees from diseases caused by CTV. Another objective is to produce useful tools for other researchers as an aid to improve citrus production and marketing in both countries. We have produced a first generation of virus-based transient expression vectors to quickly test genes in citrus trees.

Our collaborative research program has been focused, complementary, and productive. We have been effective in jointly planning experiments and methodologies and exchanging results. Although we have overlapping expertise, the Dawson laboratory focuses more on manipulation of the viral genome and the Bar-Joseph laboratory focuses more on the virus-host biology. The development of the genetic system of CTV, the infectious cDNA clone, was one example of the level of complementarity and synergism. The Dawson laboratory extended experience from TMV to CTV to obtain the cDNA clones, but the biological expertise of Bar-Joseph was needed to extend the genetic system to plants. Similar levels of synergism occurred in almost all projects as demonstrated by our publication record (Bar-Joseph and/or Mawassi from Israel and Dawson from the US are jointly on most publications).

Some other specific accomplishments are listed: (1) We developed a much better understanding of CTV defective RNAs (dRNAs) which are parasitic RNAs that are replicated with the help of the wild type virus. (2) We have identified numerous gene functions: ORF 1a and 1b are required for replication; the p33 is needed for infection of sour orange plants; the p6 gene is needed for infection of citrus trees; the p65 (hsp70 homologue), p61, p27 (minor coat protein), and p25 (major coat protein) are all required for efficient virion assembly; the p18 is needed for movement in sour orange; the p20 gene encodes the amorphous inclusion body protein and is required for infection of trees; and the p23 protein regulates the asymmetry of plus-to-minus strands of genomic and subgenomic (sg) RNAs by inhibiting minus-strand synthesis. (3) We developed an understanding of the regulation of sgRNA production. We characterized the controller elements (CE's) for the 3'-coterminal sgRNAs and for the 5'-terminal LMT RNAs. Although minimal elements for both were about 50 nucleotides consisting of two predicted stem-and-loop structures, they were distinctly different in initiation and termination of sgRNAs, perhaps suggesting different evolutionary origins.

PUBLICATIONS:

- Mawassi, M., Satyanarayana, T., Gowda, S., Albiach-Martí, M. R., Robertson, C., and Dawson, W. O. 2000. Replication of heterologous combinations of helper and defective RNA of citrus tristeza virus. *Virology* 267: 360-369.
- Albiach-Martí, M. R., Mawassi, M., Gowda, S., Satyanarayana, T., Hilf, M. E., Shanker, S., Almira, E. C., Vives, M. C., López, C., Guerri, J., Flores, R., Moreno, P., Garnsey, S. M., and Dawson, W. O. 2000. Sequences of citrus tristeza virus separated in time and space are essentially identical. *J. Virol.* 74:6856-65.
- Gowda, S., Satyanarayana, T., Davis, C., Navas-Castillo, J., Albiach-Martí, M. R., Mawassi, M., Valkov, N., Bar-Joseph, M., Moreno, P., and Dawson, W. O. 2000. The p20 gene product of citrus tristeza closterovirus accumulates in the amorphous inclusion bodies. *Virology* 274: 246-54.
- Mawassi, M., Satyanarayana, T., Albiach-Martí, M. R., Gowda, S., Ayllón, M. A., Robertson, C., and Dawson, W. O. 2000. The fitness of *Citrus Tristeza Virus* defective RNAs is affected by the lengths of their 5'- and 3'-termini and by the coding capacity. *Virology* 275: 42-56.
- Karasev, A.V., Gowda, S., Bar-Joseph, M., and Dawson, W.O. 2001. Closteroviruses. Pages 228-230, *Encyclopedia of Plant Pathology*, 2 Vol. Set. O.C. Maloy and T.D. Murray, eds. John Wiley & Sons, Inc.
- Satyanarayana, T., Gowda, S., Mawassi, M., Albiach-Martí, M. R., Ayllón, M. A., Robertson, C., Garnsey, S. M., and Dawson, W. O. 2000. Closterovirus HSP70 homolog and p61 in addition to both coat proteins function in efficient virion assembly. *Virology* 278: 253-265.
- Lee, R.F. and Bar-Joseph, M. 2000. Citrus tristeza virus, pp61-63 in *Compendium of Citrus Diseases* (Timmer, Garnsey and Graham Eds.) APS Press
- Bar-Joseph M., Che, X., Piestun, D., Botuman, O., Gofman, R., Ben Shalom, Y., Yang, G. and M. Mawassi 2001 Citrus Tristeza Virus (CTV) Biology Revisited: Quick Decline and Seedling Yellows- The Cost of Sour Orange Resistance Gene(s) *Proceedings of the International Society of Citriculture*.
- Dawson, W. O., Satyanarayana, T., Gowda, S., Mawassi, M., Albiach-Martí, M. R., Ayllón, M.

- A., and Rabindran, S. 2001. Development of a genetic system of the largest known plant virus, *Citrus tristeza virus*. Proceedings of the International Society of Citriculture.
- Satyanarayana, T., Bar-Joseph, M., Mawassi, M., Albiach-Martí, M. R., Ayllón, M. A., Gowda, S., Hilf, M. E., Moreno, P., Garnsey, S. M., and Dawson, W. O. 2001. Amplification of *Citrus tristeza virus* from a cDNA clone and infection of citrus trees. *Virology* **280**, 87-96.
- Gowda, S., Satyanarayana, T., Ayllón, M. A., Albiach-Martí, M. R., Mawassi, M., Rabindran, S., Garnsey, S. M. and Dawson, W. O. 2001. Characterization of the *cis*-acting elements controlling subgenomic mRNAs of *Citrus tristeza virus*: production of positive- and negative-stranded 3'-coterminal and positive-stranded 5'-coterminal RNAs. *Virology* **286**, 134-151.
- Che X., Piestun D., Mawassi M., Yang G., Satyanarayana T., Gowda S., Dawson W. O., and Bar-Joseph, M. 2001. 5'-Coterminal subgenomic mRNAs in citrus tristeza virus-infected cells. *Virology* **283**: 374-381.
- Satyanarayana, T., Gowda, S., Ayllón, M.A., Albiach-Martí, MR., Rabindran, S. and Dawson, WO. 2002. The p23 protein of *Citrus tristeza virus* controls asymmetrical RNA accumulation. *J. Virol.* **76**: 473-483.
- Che X, Mawassi M, Bar-Joseph M. 2002. A Novel Class of Large and Infectious Defective RNAs of Citrus Tristeza Virus. *Virology* **298**:133-45.
- Satyanarayana, Tatineni, Gowda, Siddarama, Ayllón, Maria A., Albiach-Martí, Maria R., and Dawson, William O. 2002. Mutational analysis of the replication signals in the 3'-nontranslated region of *Citrus tristeza virus*. *Virology* 300: 140-152.
- Gowda, S., Ayllón, M.A., Satyanarayana, T., Bar-Joseph, M., and Dawson, W. O. 2003. Transcription Strategy in a *Closterovirus*: A Novel 5'-Proximal Controller Element of *Citrus Tristeza Virus* Produces 5'- and 3'-Terminal Subgenomic RNAs and Differs from 3'-ORF Controller Elements. *J. Virol.* 286: 134-151.
- Che, X., Dawson, W. O., and Bar-Joseph, M. 2003. Defective RNAs of *Citrus tristeza virus* analogous to *Crinivirus* genomic RNAs. *Virology* 310: 298-309.