Remembering Steve Garnsey
Biography

Biography of Stephen M Garnsey

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Stephen (Steve) M Garnsey was born August 3, 1937 in southern California and raised on the family’s homesteaded ranch. He was educated in the one-room school nearby in DeLuz and high school in Fallbrook. He next entered the very first class along with 130 other students with 65 faculty in the newly established University of California at Riverside. As an undergraduate there, he was introduced to plant pathology by part-time employment with Pete Tsao and George Zentmyer in the University of California Citrus Experiment Station, not yet part of University of California at Riverside. Based on his farming background and advice from Zentmyer, he moved to the University of California at Davis for graduate studies in plant pathology. He was introduced to viruses working with his major professor Tom Shalla, and received his PhD working on the seasonal variation in the mechanical transmission of Tulare apple mosaic virus (Garnsey 1963).

Upon receiving his degree at Davis in 1964, he accepted a position with the United States Department of Agriculture (USDA ARS) in Orlando, Florida. Initially, he began examining virus-like diseases of citrus beginning with work on tatter leaf, crinkly-leaf, infectious variegation, and exocortis viruses. (At this time, all graft-transmissible diseases were considered viruses, as viroids, phytoplasmas, and systemic bacteria had not yet been discovered.) One of his first important achievements was his demonstration that exocortis could be spread by mechanical transmission via contaminated tools used in citrus, which became the basis for future control of exocortis (and other viroids) spread by disinfesting tools in the greenhouse and in the field with diluted bleach (Garnsey and Jones 1967).

Steve’s research soon evolved into the purification and characterization of viruses of citrus, which culminated into a very productive relationship with Dennis Gonsalves who had just accepted a position nearby at the University of Florida Citrus Research and Education Center at Lake Alfred. Steve had already developed effective methods for purifying these viruses that had multi-nucleoprotein components. While collaborating to investigate the infectivity of the separated RNA components, they showed that non-infectious mixtures of RNA components became infectious by adding small amounts of purified coat protein to the RNA mixture (Gonsalves and Garnsey 1975a, 1975b, 1975c). This phenomenon of “protein activation” had only just recently been discovered with alfalfa mosaic virus. Importantly, they would expand their collaboration to citrus tristeza virus (CTV), arguably the most important virus of citrus.

In 1973, Steve took a year sabbatical at the University of Florida in Gainesville, where the collaboration with Dennis increased to include Dan Purcifull. There he was able to increase his skills including electron microscopy (EM) of virions in extracts, detection of virus inclusions by light microscopy of stained tissues, serological techniques, along with transmission of plant viruses by insect vectors. Steve used the EM tissue dips to determine that young flushes of CTV infected plants had high levels of CTV particles. He subsequently grew CTV infected plants in Orlando greenhouses and weekly sent “care packages” of young CTV infected shoots to Lake Alfred, from which Dennis Gonsalves extracted and purified CTV over a period of several months (Gonsalves et al. 1978). Dan Purcifull at Gainesville subsequently used the purified CTV to prepare specific antisera in rabbits. Steve showed that the antisera was highly effective to diagnose CTV in citrus by immunodiffusion tests in the presence of sodium dodecyl sulfate, which degraded the elongated CTV into antigenic components that diffused and reacted in agar gels (Garnsey et al. 1979). Thus, CTV could now be detected in citrus in two days as compared to 8 months by biological indexing on Mexican limes. About the time the CTV antisera were being prepared and tested, Michael Clark and colleagues in England were developing the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) for sensitive, specific detection of plant viruses. Steve attended a workshop on ELISA techniques and returned to successfully apply ELISA for detection of CTV in citrus. In cooperation with Moshe Bar-Joseph and his colleagues in Israel, the CTV antiserum was used in ELISA for large-scale, rapid and sensitive CTV detection (Bar-Joseph et al. 1979). The significance of the CTV serodiagnosis was recognized in 1981, by the presentation of the American Phytopathological Society’s Lee M Hutchins award jointly to Moshe Bar-Joseph, Michael
Clark, Steve Garnsey, Dennis Gonsalves, and Dan Purcifull.

In 1978, Steve started a collaboration with a new research group working on citrus virus diseases and their control at the INIA station in Moncada, Valencia (Spain), later called IVIA, that would continue throughout his career. A loan from the World Bank for the development of agricultural research in Spain allowed: i) Mariano Cambra to learn serology and immunoenzymatic techniques at the Station de Pathologie Végétale de l’INRA, Angers, France; ii) Pedro Moreno to obtain his MSc in Plant Pathology and learn about citrus virus diseases at the University of California at Riverside; and iii) Luis Navarro to develop the technique of shoot-tip grafting in vitro to obtain virus-free citrus plants in T Murashige’s lab, also at the University of California at Riverside. Through Pedro and Luis, Steve was introduced to Mariano Cambra to whom he provided his anti-CTV antiserum 879 (probably the best ever developed) to this group for quick detection of CTV, which in those years was ravaging citrus plantings on sour orange rootstocks in Spain. In 1979, ELISA detection of Spanish CTV isolates was published at the same time as detection of Florida CTV isolates. At that time Steve was the Chairman of the International Organization of Citrus Virologists (IOCV) and at the 8th Conference of the organization held in Australia, Luis Navarro and Pedro Moreno publicly thanked him for sharing his antiserum, updated him on the results obtained, and offered him co-authorship of their paper. However, Steve declined this offer as he had already submitted his own manuscript on ELISA for CTV detection. Availability of the 879 antiserum and ELISA for CTV detection was most helpful for purification of a Spanish CTV isolate to obtain the first monoclonal antibodies to CTV. The 879 antibody also allowed surveys and studies on CTV epidemiology in several regions of Eastern and Southern Spain.

Mariano Cambra and colleagues in Spain produced the first monoclonal antibodies to CTV followed by monoclonal antibodies production by Tom Permar and Steve in Florida along with David Gumpf in California (Permar et al. 1990), Mei-Chen Tsai et al. in Taiwan, Mohamed Zebzami et al. and Lochy Batista et al. in Cuba. Instead of all groups competing, Steve initiated the creation of the “CTV Mabs friends informal team” that established a collection of CTV-specific Mabs at Steve’s USDA ARS Orlando laboratory and at IVIA in Spain and they were tested against the international collection of CTV isolates at the USDA ARS quarantine facilities at Beltsville.

While working with Dennis Gonsalves on mechanical transmission of exocortis by stem slashing, Steve and Dennis made a fortuitous discovery that became the basis for further progress with CTV. In examining Mexican lime seedlings stem slashed with field samples of exocortis, Steve noticed CTV-like symptoms in some of the resulting plants. Upon further examination, he found that these trees had become infected with CTV (Garnsey et al. 1977). Although many field samples were infected with both exocortis and CTV, the dogma was that a phloem-limited virus like CTV could not be mechanically transmitted. So much for dogma. Further work developed this into a routine procedure to transmit this virus. Much of the work that was accomplished with CTV over the next 30 years could not have been done without this seminal discovery.

As more researchers moved into Florida, Steve developed more collaborations. He worked with Pete Timmer on citrus ringspot virus and Richard Lee and Ron Bransky in improvement of purification and detection of CTV. The collaborative studies on citrus ringspot yielded interesting results that later would be important to unravel the etiology of this disease. Steve and Timmer transmitted ringspot to some herbaceous hosts, mainly to Chenopodium quinoa and Gomphrena globosa, in which the virus caused local lesions. Moreover, they made serial single-lesion transfers to new G. globosa plants in an attempt to biologically clone the ringspot virus, then mechanically transmitted the biologically cloned virus to ‘Etrog’ citron, and then by graft to sweet orange plants. Although ringspot in Florida showed some differential symptoms, it was always associated with severe bark scaling, the characteristic symptom of psorosis. The sweet orange plants inoculated with the biologically cloned ringspot showed the same bark scaling as the original isolate, thus excluding a contaminant virus as the cause of the local lesions observed, and strongly suggested that psorosis and ringspot were likely induced by the same virus. Local lesions from G. globosa were later used for partial purification and characterization of Citrus psorosis virus.

Steve recruited Ray Yokomi to the USDA ARS lab in Orlando who used aphid transmission to separate components of CTV populations from each other as well as other graft-transmissible citrus pathogens. “Cleaned up” CTV isolates as well as wild-type field isolates (identified by a FS-number) were graft-inoculated in a citrus host range to determine their phenotype. Characterized isolates were assigned a “T” number (e.g. T30, T36, T54, etc.) and selected for genetic characterization, cross-protection, and other studies. Vector studies included the brown citrus aphid (Toxoptera citricida), the cotton or melon aphid (Aphis gossypii) and the green citrus or spirea aphid (A. spiraeola) and confirmed that the brown citrus aphid was the most efficient, hence, important vector of CTV. This data would prove vital in later studies that defined CTV epidemiology was dependent on the predominant vector species and its seasonal phenology along with the dominant CTV strain present. Hence, managing CTV was found to be different from region to region and host to host.

Steve began collaborating with Tim Gottwald soon after Gottwald transferred to the USDA ARS Orlando lab in 1985 and their collaboration on the epidemiology of citrus diseases persisted throughout the remainder of Steve’s career with ARS. Together they designed and established a federal research project on domestic, exotic and emerging diseases of citrus that is the enduring foundation upon which USDA citrus pathology research is built.
Prior to Gottwald’s arrival, Steve and others had drafted a Citrus Canker Action Plan for Florida should the pathogen be introduced, which it was soon thereafter. Gottwald’s main assignment was to examine the epidemiology and control of citrus canker, which was a quarantine pathogen that could not be studied in Florida. So, Steve and Gottwald packed up several hundred potted citrus trees and shipped them via refrigerated semi-truck to the USDA, Foreign Disease-Weed Science facility located on Ft. Detrick in Fredrick, Maryland over two seasons. There, along with Ed Civerolo, they planted several field plots that they inoculated with Xanthomonas citri pv. citri under quarantine and more than 800 miles north of all citrus production areas. They continued this project for several years reestablishing the plots each spring followed by mathematically describing the spatial and temporal spread of the disease. This work was part of the body of work that eventually distinguished a second bacterial disease of citrus, Citrus Bacterial Spot (CBS), from Asiatic Citrus Canker (ACC), cleared up the confusion between the diseases and resulted in the deregulation of CBS and refocus on the more serious pathogen, ACC (but resulted in Gottwald having to testify in court about “junk science”).

Throughout the 1990’s, Steve and Gottwald worked on multiple aspects of CTV increase and spread. Along with Mike Irey they monitored and described the spread of CTV in South Florida commercial groves prior to the introduction of the T. citricida. Turning to the Dominican Republic, they monitored and described the rapid dissemination of mild isolates of CTV following introduction of T. citricida in various locations within the Dominican Republic. They also followed the evolution and spread of a severe stem pitting isolate of CTV in the Dominican Republic. As described below, Steve and colleagues had developed a differential monoclonal antibody system to differentiate strains of CTV. Gottwald and Steve used this differential system to track the co-diffusion of severe versus mild strain isolates of CTV in multiple locations in Costa Rica when T. citricida was newly introduced there. In a series of subsequent studies Gottwald and Garmsey worked with Spanish and Dominican Republic collaborators and was able to tease out the differential effects on T. citricida versus the cotton aphid, A. gossypii, on temporal increase and spatial patterns of spread of CTV. As it turns out the cotton aphid is a migrating aphid species that spreads CTV over long distances and establishes new foci of infection, whereas, T. citricida is a colonizer of citrus and spreads CTV within individual plantings. The team demonstrated that when these two aphid species occur in the same area, they are a devastating duo that inadvertently cooperate to spread and increase all CTV isolates causing rapid epidemics. This led to several seminal stochastic modeling papers on vector/disease dynamics.

Steve and Gottwald traveled to Japan and Taiwan together on multiple occasions examining a number of citrus diseases. They become interested and involved in citrus huanglongbing (HLB) quite early. While in Taiwan, Gottwald and Steve joined with Prof. Hong Ji Su in establishing research plots to examine the spread of HLB in mixed mandarin and pummelo blocks. Over the next five years, they monitored the spread of an HLB isolate through the mandarin trees in the plantings. Toward the end of the project, they discovered and monitored a second isolate of HLB spreading through the previously uninfected pummelo trees. This work demonstrated the diversity of HLB isolates and complexity in HLB epidemics. Steve and Gottwald wrote articles in the 1990s on the diversity of foreign citrus diseases that may eventually threaten the US citrus industries and particularly warned of the potential for HLB to be introduced to the US and possible consequences.

CTV in Florida caused decline and death of trees on the sour orange rootstock. However, not all isolates of CTV caused this disease. Some isolates of CTV had no effect on trees on the sour orange rootstock. One of the epic developments in citrus pathology was the development of a monoclonal antibody to CTV (MCA-13) that was able to discriminate decline isolates of CTV from isolates that did not affect trees on the sour orange rootstock done with Tom Permar and David Gumpf (Permar et al. 1990). This antibody became the major tool for managing CTV in Florida. Trees that were MCA-13 negative were allowed to be propagated in Florida. MCA-13 was also provided by Steve to his colleagues in Spain to test this antibody with CTV isolates in Spain, where the association of the MCA-13 reaction with isolates causing decline of trees propagated on sour orange was not as strict as in Florida. Later, Mariano Cambra would visit Steve’s lab in Orlando to compare serological reactions of different CTV isolates with antisera 879, MCA-13, and a wide panel of Spain and Florida monoclonal antibodies that revealed a wide diversity of serotypes among CTV isolates. This collaboration also resulted in the development of the direct tissue blot immunoassay (DTBIA) for quick detection of CTV, a technique very adequate for sample processing without any need for extract preparation, that enabled mailing imprinted membranes for processing in a distal laboratory, or store membranes for years at room temperature before processing with newly developed antibodies or probes. CTV detection had become even more simple and efficient.

A major accomplishment was the development of the collection of exotic citrus diseases in Beltsville, Maryland. The literature on CTV from different countries had been very confusing, with different isolates in different countries inducing a wide range of symptoms. Since no citrus industry would allow the import of CTV isolates from other countries, the different isolates could not be directly compared. It was not known how much of the symptom variation was due to environment and the particular citrus host grown in the different countries. Steve, along with Ed Civerolo, David Gumpf, Richard Lee, Ron Branski, Ray Yokomi, and John Hartung established a collection of exotic citrus diseases and pathogens from around the world in a greenhouse more than 800 miles away from commercial citrus. Many collaborators from around the
world (Argentina, Brazil, China, Colombia, Dominican Republic, India, Israel, Italia, South Africa, Spain, Turkey, Venezuela and others) contributed to this collection with locally characterized CTV isolates, psorosis or psorosis-like isolates, citrus variegation, tatter leaf and other citrus diseases. For the first time, this allowed the direct comparison of different pathogen isolates from all over the world. With the addition of Mark Hilf to Steve’s lab, they documented the variability among CTV isolates based on symptoms (Garnsey et al. 2005), molecular studies showing prominent genetic divergence within the CTV groups, and differences in host resistance. Systematic biological and molecular comparisons of isolates from the different countries provided an overview of the total variation among CTV isolates and the characteristics of CTV populations in specific areas. The variability of symptom severity (isolate and host specific), and isolate-specific host range differences all suggested a complex origin for the current global population of CTV in commercial citrus. Similarly, the CTV isolates from 20 different countries were used to define genotypic groups of CTV containing similar sequences as “strains” and additionally the importance of recombination in CTV evolution (Hilf and Garnsey 2000).  

Infected tissue from this collection was available for research purposes to people in other countries. For example, psorosis-like isolates from different countries provided to the IVIA group in Moncada enabled the development of reagents for detection by ELISA, molecular hybridization, RT-PCR and immunoelectron microscopy, and analyzing genetic diversity and evolutionary forces shaping populations.

Steve met Bill Dawson at an American Phytopathological Society meeting about 1990, where Dawson brought up the idea of making an infectious cDNA clone of CTV to allow reverse genetic analysis of the virus as his laboratory had previously done with TMV. Steve was skeptical at first, but upon further reflection came up with some funding to start the process, which resulted in hiring Alexander Karasev to begin cloning. Dawson had wanted to work with CTV in California, but funding for CTV research there was owned by other researchers. About this time, Steve told him about a position in Lake Alfred, FL, and encouraged him to apply. Since this would allow obtaining enough funding to support the cloning of CTV, Dawson moved to Florida and established a life-long partnership with Steve. Steve knew citrus, citrus viruses, purification, immunology, detection, and CTV interactions with its hosts. Dawson knew cloning and basic virology. This partnership, which was almost totally complementary, continued throughout their careers.

One of the first things that Steve did was to teach Cecile Robertson how to grow and maintain citrus for the Dawson laboratory. He was a great teacher as exemplified by the fact that he was the only person that could get by with calling Cecile “Sweety”. His directions still guide the greenhouse work on CTV at Lake Alfred.

At that time, the University of Florida team of Richard Lee and Chuck Niblett had begun sequencing the CTV genome. This effort was led by Hannu Pappu, who was sequencing three large clones obtained by a former student, Lee Calvert. Meanwhile, Ken Kline contacted Steve for a source of CTV because he wanted to clone the coat protein gene in order to attempt to produce transgenic citrus to be resistant to CTV, which was the standard protocol for developing resistance to viruses at the time. He cloned the gene in an Escherichia coli expression system that allowed the identification of the correct clone by detection with available coat protein antiserum. This turned out to be one of the luckiest breaks for the future of CTV work because Steve gave him plant tissue infected with the T36 strain of CTV, which much later would be discovered to be by far the easiest strain of CTV to clone. The Kline laboratory successfully cloned the coat protein gene and its sequence was the first sequence of CTV. Meanwhile, the sequencing of the three large clones by Pappu did not appear to represent viral clones, so the laboratory decided to start sequencing by walking from the Kline coat protein sequence, which resulted in sequencing of approximately 7000 nts of the 3’ end of the genome. The completion of the 19,296 nt genome sequence was led by Alexander Karasev.

With the sequence completed, the next step was to build an infectious cDNA clone, which involved Satyanarayana Tatineni, Siddarame Gowda, and Vitaly Boyko. With the expectation that with an infectious clone of CTV, there would need to be some way to infect citrus seedlings with RNA transcripts or the cDNA, Steve along with Mark Hilf began working on inoculating citrus seedlings with viral RNA. Steve and Dennis Gonsalves had demonstrated that young citrus seedlings could be infected with CTV if partially purified virions were applied to the edge of a scalpel which was used to slash cuts into the stem of the seedling, introducing virions into the wounds, leading to a systemic infection. Mark and Steve had found that a biolistic approach also was an effective method to introduce virions into citrus seedlings, leading to systemic infection. They cut patches of bark from the trunk of a citrus seedling and applied a virion preparation to the inner surface. Tungsten pellets were then shot into the inner surface of the bark patch under partial vacuum inside a biolistic chamber. The bark patch was then replaced on the tree and allowed to heal. The wounds caused by the tungsten pellets created entry points for the virus, which caused a systemic infection once the bark patch successfully grafted. Excitement about the possible introduction of virion RNA into tissue using biolistic inoculation was soon dampened when repeated attempts with virion RNA did not mimic the success achieved with virions.

Construction of a 20,000 nt cDNA that was infectious was a difficult task, but it was possible because of some lucky breaks. First was Steve’s choice of giving CTV strain T36 to Ken Kline to initiate sequencing. It turns out that this was the only single sequence isolate available in the collections making cloning much simpler. Sequences of CTV within the replicate genes are toxic to E. coli. The second lucky break was Tatineni’s realization that correct
clones came only at two or three days after transformation. The third lucky break was the discovery by Silvia Ambros in Pedro Moreno’s lab that CTV could spread systemically in *Nicotiana benthamiana* after agro-inoculation. However, the most important fortuitous development was Steve and Gonsalves’ discovery that CTV could be transmitted to citrus by slashing stems with virions. Otherwise, the choices would have been extraordinarily difficult since aphid transmission after membrane feeding and direct agro-inoculation of citrus have not worked so far.

Once the infectious clone of CTV T36 was accomplished, Steve guided the biological experiments. First was showing that the p23 gene and 3’ non-translated sequences were sufficient to induce ‘seedling yellows’ symptoms by Remei Albiach-Martí. Then the deletion of CTV genes to identify what genes were needed to infect citrus plants, the effect of gene deletions on the host range of the virus, and the effect of deletions on the production of stem pitting in citrus led by S Tatineni. Actually, the results were a surprise. Steve designed the original experiment with the expectation that prior infection with the deletion mutants, which were expected to be milder than the wild type virus, would cross-protect trees and prevent the wild type virus from causing a mild stem pitting. The result was that some of the deletion mutants induced much more severe stem pitting than did the wild type virus. The dogma at the time was that some particular sequences of the CTV genome would be correlated with stem pitting and there were several funded sequencing projects to identify “stem pitting” sequences so that they could be used to identify severe isolates of CTV. However, in this case, it was the removal of sequences that induced stem pitting.

Steve was also instrumental in designing the cross protection experiments that demonstrated that only isolates within a strain could cross protect against each other and the mapping of sequences were also involved in inducing decline of sweet orange on sour orange rootstocks. With strain T36, the p23 gene products was found to be involved in induction of both seedling yellows and decline on sour orange.

In 2000, after 36 years working with the USDA-ARS as the laboratory was moving from Orlando to Fort Pierce, Steve retired. However, this did not stop the partnership with the Dawson laboratory, where he became a Visiting Scientist with the University of Florida. Initially from Orlando and eventually from his ranch in California, he still guided the CTV biological experiments.

While in California Steve, partnered with Dave Gumpf and Joe Semancik at UC Riverside to advise Georgios Vidalakis, current Citrus Clonal Protection Program (CCPP) director, in his PhD studies. Dave actually wrote to Georgios in 1999 that one of the reasons he was accepting him as his student, even though he was planning to retire from CCPP very soon, was that “arguably the best citrus virologist in the world was retiring from USDA-ARS in Florida” and he was moving into his family’s ranch, not far from Riverside, and he was planning to engage him in Georgios’ PhD program. After the untimely death of Dave in February of 2003, Steve accepted an appointment with UC Riverside to serve in Georgios’ PhD committee and guided the work on the efficacy of biological indexing of graft-transmissible citrus pathogens in mixed infections, which brought together information fragmented in many older publications and added statistical analyses to information previously only treated anecdotally (Vidalakis et al. 2004). It also demonstrated that different CTV genotypes interacted differently with other citrus viruses like in the case of CTV T30 isolates that suppressed vein enation symptoms in Mexican lime. For 18 years after his retirement, Steve worked tirelessly to support the citrus industry and research in California. He served in numerous committees and as the public member of the Citrus Research Board (CRB) and he remained to the end an invaluable resource and advisor to the Central California Tristeza Eradication Agency, the USDA-ARS National Tristeza Eradication Repository for Citrus and Dates and the CCPP.

Steve was instrumental in taking citrus virology from the beginning of purifications and characterizations through the reverse genetics defining the structure-functions of CTV to the development of the virus into a vector as a tool to improve citrus and combat other diseases. Considering all these contributions and his participation in the IOCV, he was elected Fellow of the International Organization of Citrus Virologists. In addition to his many scientific accomplishments, Steve made many more significant contributions that are hard to quantify but significant none the less. Steve was a mentor, a teacher, and a friend to many. Through his many interactions, he helped shape the careers of many young scientists and helped keep the old ones on track. He was never at a loss for ideas and was one of the best sounding boards that a fellow researcher could have. He could talk at all levels, always positive, and when you walked away, you left with more than you came with. Gottwald who had the privilege to work with and travel with Steve extensively summed him up for many colleagues as, “The most valuable of colleagues, congenial and generous traveling companion, mentor, consummate character, much needed humorous distraction (a cross between Gary Cooper and Dave Barry), and a deep personal friend”. However, Steve could not make a long story short.

References


Getting started

In the field

And the greenhouse

Collecting data

Receiving awards

USDA retirement

Next career at UF

Making a long story short