

# INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

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IOCV NEWSLETTER

April, 2006

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## FROM THE CHAIRMAN

## Difficult times

2006 is now well underway, so let me begin with a slightly belated Happy New Year to everyone. Hopefully you will all have seen the IOCV website now functioning courtesy of IVIA ([www.ivia.es/iocv](http://www.ivia.es/iocv)). In addition to information about IOCV, its members and newsletters, both the 14<sup>th</sup> and 15<sup>th</sup> Proceedings are available there in pdf format. We hope to have other proceedings on-line in the near future.

Talking of Proceedings, Mark, Nuria and Mario have been busy putting the final touches to the 16<sup>th</sup> Proceedings.

You should all have received the 1<sup>st</sup> announcement of the 17<sup>th</sup> conference from Nukut Onelge. To remind you, it will be held in Adana in October, 2007. She and her colleagues are arranging some interesting tours, so please do all you can to be there – remember, IOCV will be celebrating its 50<sup>th</sup> anniversary. Send her your positive responses so she can make the necessary arrangements, and pass on the notice to non-members who have an interest in graft and insect transmitted citrus diseases. The announcement has also been posted on the webpage.

We also need to be thinking of possible venues for the 18<sup>th</sup> conference in 2010. The earlier we have official invitations, the better.

Progress is also being made in the updating of the IOCV slide collection, which will be on-line. Josy Bové, Pedro Moreno and Nuria Duran-Vila, who are taking the lead in this project, met recently in Moncada and prepared an outline of the new collection. You may get a request to contribute, and your assistance will be most welcome.

Along with the above positive developments, humans and nature continue to present new challenges. Huanglongbing has now been shown to be present in several counties in Florida, and the International Canker Symposium in Orlando last November was expanded to include HLB – several IOCV members from the USA and overseas participated. The Florida situation has prompted other citrus growing states in the US to have a close look at their trees to see if the disease is present. Texas, which already has the Asian citrus psyllid, is on high alert. Brazil is now deeply involved in HLB research and control, and will host an international symposium on the subject this July (see [www.huanglongbing.com.br](http://www.huanglongbing.com.br)).

Good communications and collaborations between IOCV members continues, and if you find anyone interested in graft and arthropod-vectored pathogens who is not an IOCV member, please give them an application form.

John da Graça

These are difficult times for the citrus industries of the world. Unlike other fruit crops, there are 8 to 10 diseases of citrus which are extremely destructive and can debilitate a citrus industry. Two of these diseases, tristeza and huanglongbing (HLB - greening disease) now are serious threats to major citrus industries throughout the world.

As indicated and illustrated by Nolasco (Pg. 9) *Toxoptera citricida* is now well established in northern Portugal with CTV reported present. We observed an abundance of back yard trees throughout northern Portugal and can presume that these back yard trees also exist in northern Spain where *T. citricida* is present (see Ecoport slide show No. 194 on observations of *T. Citricida* in Northern Portugal). *T. citricida* is also present throughout the Caribbean, Central America, Mexico and Florida and poses a severe threat to the citrus predominantly on the sour orange rootstock in most of these regions. Guardo et al. (Pg. 4) discuss the tristeza problem in parts of Italy and McConnell and Moody (Pg. 4) discuss stem pitting tristeza problems in Jamaica.

HLB (greening) - one of the most devastating diseases of citrus is on a worldwide destructive rampage as indicated in the abstract by Bove (Pg. 2). A number of inputs to this newsletter suggested severe problems with HLB worldwide as in Brazil by Eduardo Carlos (Pg. 2) and by Lopes (Pg. 9). Argentina is now gearing up for possible entry of the greening pathogen (Costa and Plata (Pg. 8) as is Texas (da Graça Pg. 10). In Florida, a recent article in the Florida Citrus Industry magazine indicated that "Florida has thrown in the white flag" (Pg. 3). Kesinger reports on the role of the Florida Citrus Plant Protection Committee in the challenges ahead for Florida by the threat posed by greening disease (Pg. 8). Inputs by Le Roux (Pg. 5); Van Vuuren (Pg. 7) and Pietersen (Pg. 6) indicate how South Africa is handling the greening problem. Ochasan indicated what is being done in the Philippines in living with HLB (Pg. 5) and Le Thi Thu Hong reports on the difficult problems they are facing with HLB in Vietnam (Pg. 7).

With the threat of the spread of *T. citricida* into the countries of the Mediterranean basin, where most of the citrus industries are still on the sour orange rootstock, there is the potential of a repeat of the tristeza epidemics which occurred in Argentina and Brazil in the 1940's. With the presence of the vector *Diaphorina citri* in Saudi Arabia, Yemen and Iran, HLB is poised to enter the countries of the Near and Middle East. *D. citri* is currently abundant in the spring flush of citrus in Mexico and Central America and can be compared to waiting for a match (the HLB bacteria) to inflame all of the countries of the region, including Texas.

Faced with these diseases (not mentioning sudden death disease, blight, canker, citrus variegated chlorosis, leprosis etc.) difficult times are ahead for the citrus industries of the world.

C.N. Roistacher and John daGraça

## Y.S. Ahlawat - India

Indian Agricultural Research Center, New Delhi  
Ahlawat retires.

Dear Friends/Colleagues, My best greetings for Dipawali -- I wish to convey my sincere thanks for a long association, your kind help and support all these long years. Although I shall be superannuating on 31.10.2005 a day before Dipawali so that the festival is celebrated in a nice way. I shall remain in touch with you and looking forward to your kind help, best wishes and blessings in the near future.

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## Bernard Aubert - France

Aubert on Feb. 26<sup>th</sup>, 2006 wrote: In spite of a time schedule largely dedicated to local priorities, I visited Tunisia twice recently on the invitation of Mustapha Lasram former Director of the Mediterranean Institute (CIHEAM-IAMM). The objective was to join a team ( Citrus +) in charge of an intensive survey of Tunisian citrus orchards and nurseries, and submit a report with recommendations to local authorities. Preventing the spread of citrus diseases and pests, developing new rootstocks and certified scion cultivars, proposing related strategies for the renewal of old orchards, were the main aspects investigated.

Bernard also wrote about his work on coastal pollution of Mediterranean waters:

The problem is addressing the pollution of wastewater in our coastal environment especially from the urban area of Montpellier. I am coordinating NGOs monitoring the discharge of pollutants in coastal waters with a view of keeping high quality grade of beaches, fishing areas, and shellfish production.

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## J.M. Bové - France

INRA /Université de Bordeaux

Abstract of: "Huanglongbing: a destructive, newly-emerging, century-old disease of citrus" by Joseph M. Bové, Journal of Plant Pathology, 2006, 88: 5-35.

A detailed account is given of the history, aetiology, biology, epidemiology, detection, geographical distribution, and control of huanglongbing (HLB), a destructive disease of citrus that represents a major threat to the world citrus industry, and is slowly invading new citrus-growing areas. HLB, whose name in Chinese means "yellow dragon disease", was first reported from southern China in 1919 and is now known to occur in next to 40 different Asian, African, Oceanian, South and North American countries. The agent is a phloem-restricted, non cultured, Gram-negative bacterium causing crippling diseases denoted "greening" in South Africa, "mottle leaf" in the Philippines, "dieback" in India, "vein" phloem degeneration" in Indonesia. The HLB bacterium belongs to the genus *Candidatus Liberibacter*, three species of which are currently known, *Candidatus Liberibacter asiaticus*, occurring in Asian countries and, to a lesser extent, in Brazil and the USA (Florida), *Candidatus Liberibacter africanus* with its subspecies "capensis", recorded from African countries, and *Candidatus Liberibacter americanus* present in Brazil. The suggestion is that each liberibacter species has evolved in the continent after which it is named. HLB symptoms are virtually the same wherever the disease occurs. Infected trees show a blotchy mottle condition of the leaves that results in the development of yellow shoots, the early and very characteristic symptom of the disease. Trees are stunted, declining

and bear a few, small-sized, and deformed (lop-sided) fruits, that are poorly coloured (greening) and with coloration starting at the peduncular end (colour inversion). Aborted seeds are characteristic of affected fruit. HLB can be transmitted by grafting from citrus to citrus and experimentally by dodder to periwinkle. The psyllids *Triozia erythrae* and *Diaphorina citri* are natural vectors. Two different types of HLB are known, the heat-sensitive African form transmitted by *T. erythrae*, which develops at temperatures of 22-25 °C, and the heat-tolerant Asian form, transmitted by *D. citri*, which stands temperatures well above 30 °C. Although the HLB pathogen can be identified by electron microscopy, other laboratory methods are used for routine detection. ELISA with monoclonal antibodies is not recommended. Better systems are dot blot hybridization with a DNA probe, and various PCR formats (one-step, nested, multiplex) using species-specific primers based on 16S rRNA or *rplK**AJL-rpoBC* operon sequences. Because no curative methods of HLB are available, control is preventive and largely based on inoculum elimination by removal of infected trees and chemical treatments against vectors. Strict quarantine measures must be implemented to impair further international spread of HLB agents and their vectors.

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## J. Allan Dodds - California

University of California - Riverside

ProMED-mail Plant Disease Moderator

I am the plant disease moderator for ProMED-mail (<http://www.promedmail.org>) which is a disease alert system run by the International Society for Infectious Diseases. I believe this is a service some IOCV members may find useful. One of my jobs is to try and encourage more plant pathologists to subscribe. So I ask "would you be willing to subscribe to the plant disease email service at ProMED-mail?". The basic subscription is free (see left side panel of the web page, select the plant option). If you could help me spread the word in your country/region or elsewhere, please do so, and accept my thanks in advance. It is a good resource, I think. You can find all past plant disease postings by using the search term dh or jad (these are Dick Hamilton the former moderator, and myself since Jan 2006) after setting a date bracket in the search engine that is part of the archive link. By browsing you will rediscover that Plant Pathology is not a dying field. If you like it consider the four S's, Subscribe, Search, Submit and Spread-the-word. Very few plant pathologists submit, unlike on the medical side. I encourage you to cut and paste this paragraph to others. Thanks. [dodds@ucr.edu](mailto:dodds@ucr.edu) OR [dodds@promedmail.org](mailto:dodds@promedmail.org)

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## Eduardo Fermio Carlos - Brazil

Centro APTA Citros Sylvio Moreira

HLB (Greening) in Brazil

About greening.....It is being treated as a serious problem in Brazil. At the beginning of 2005, it was necessary to tie everything together under the law. After that, a multi-institutional "task force" was assembled and the eradication program started. Right now, the goal is restrain greening, because real eradication seems no longer possible. But the program has apparently being effective and the scenario today is not catastrophic. Lots of energy in many fronts has been applied; from educating and training programs for growers and inspectors to diagnosis research.

Officially as of February, 2006 about 141,000 trees were diagnosed with greening and they were either already pulled out or are in the process of being pulled. This is an ongoing process and those numbers vary weekly. But in addition to that, there is a voluntary elimination of trees done by more conscious growers, and we don't have numbers for that. By far, most of growers that had trees examined by the "Task Force" understood the situation, and just eliminated positive trees. Very few cases are still in debate.

More conscious growers did not wait for the official "Task force". It is very possible that they have pulled out around the same amount as we did, or another 140,000 trees. But there is no official report on that yet. We are organizing a second inspection for the whole São Paulo State, and then, we may have a better estimation in a couple of months. Therefore a reasonable number is around 280 thousand trees, to date.

Helvecio and I set up experiments to study the distribution of the bacterium and the characteristics of foliar symptoms in sweet orange. The results were interesting, and after a couple of thousand PCR reactions we know exactly where to collect and what type of leaves we want. Those have the highest probability of having the bacterium. The correlation is excellent and goes to 97%, giving us confidence on what we are doing. This information was presented to the growers in many commercials, trainings and seminars. So, when they find the characteristic leaf symptoms, they pull the tree out. Of course, some mistakes can be made, but considering the disease we dialing with, we think is better to be fast than 100% accurate.

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## Florida throws the white flag on greening

By Kevin Bouffard

From the Florida Citrus Industry magazine -

November/ December, 2005

Florida's war against canker went on for 10 years before a controversy arose over whether eradication was possible. It took just two months after finding the first case of citrus greening in Florida for state - and federal agriculture officials to conclude eradication of that bacterial disease is impossible.

There was a consensus of the scientists that the estimated length of time of the disease's presence in Florida, difficulty of detecting infected trees in a timely manner, movement by a small innumerable insect vector and limitations of diagnostic tools precluded making eradication of the disease feasible," according to a statement released Oct. 31 by the Florida Department of Agriculture and Consumer Services. That consensus was reached in an Oct. 20 teleconference among scientists and officials with the U.S. and state agriculture departments, the statement said.

"It's probably the most devastating disease of citrus we know of," said Timothy Gottwald, a plant pathologist with the U.S. Department of Agriculture lab in Fort Pierce.

The greening bacteria was probably introduced into the state around Homestead through infected plant material imported from areas where the disease is prevalent, including Asia, Africa and Brazil, Gottwald said. That can't be proven because the original source of infection probably died years ago. The greening bacteria can live in a plant for up to three years before symptoms appear,

"Once the symptoms show up, it's too late to save the tree," said Kuang-Ren Chung, a pathologist at the Citrus Research and Education Center in Lake Alfred. "Eventually, it is fatal."

The spread of the disease in Florida follows patterns in

other parts of the world. "The long length of time for disease symptoms to become visible and the long distance and rapid movement of the disease's bacterial causal agent by its insect vector, the Asian citrus psyllid, has led to a widespread establishment of the disease within just a few years," said the Oct. 31 statement.

By the time a state official discovered the first greening case near Homestead at the end of August, the disease had clearly spread across the state. Once established, the most common means of spreading the disease is the citrus psyllid, an insect that also serves as a host for the bacteria. The psyllid had already established itself across the Florida citrus belt since it was first discovered in June 1998 in Del ray Beach.

The initial Homestead discovery led to another nearby and a third in Pinecrest, about 40 miles to the north. That led to a survey for the disease in an area of about 150 square miles in south Miami-Dade County. Surveyors jumped five miles to the north with each new greening discovery. "Every time we jump, we find it," said Craig Meyer, the deputy agriculture commissioner in Florida.

By the end of October, state officials had confirmed more than 400 greening outbreaks in more than 265 locations across eight counties, said Denise Feiber, a spokeswoman for the state agriculture department. Those counties are Miami-Dade, Monroe, Broward, Martin, Palm Beach, St. Lucie, Hendry and Sarasota. That included two commercial groves, including one of the state's largest growers. Greening was found over a four-square-mile area in a Hendry grove owned by Southern Gardens Citrus Processing Corp. of Clewiston. Southern Gardens has about 30,000 acres of citrus grove. The disease was also found in two citrus trees five miles apart on land owned by Gallery Judge Grove of Loxahatchee in north Palm Beach County.

Given the long war with canker, growers reacted with alarm about the greening threat. "As bad as canker is, greening is worse," said Steven Rogers, a partner in Rogers Brothers Fruit Co. Inc. in Highland City and a citrus industry consultant with a doctorate in plant pathology. "It's easy to see 10 percent of Florida's groves will be eradicated because of canker. The question is: Can we afford another 10 percent from greening?"

"From what we've seen of the impact in Brazil, it's more deadly to the citrus industry than canker is," said Andy LaVigne, the chief executive at Florida Citrus Mutual in Lakeland.

Citrus officials abandoned the eradication strategy for greening before drawing up a battle plan. The scientists and regulatory officials agreed that much remains unknown about this disease. Although well-distributed throughout the citrus growing areas of the world, many key aspects of the disease and how to best manage it are still little known," according to the state agriculture department statement.

Officials will continue to track greening's movement across Florida while they develop best management practices to control the disease's impact on the citrus industry. Once developed, the agriculture department will promote the BMP through an extensive education program for growers.



**M. Guardo, G. Sorrentino and A. Caruso.**

**C.R.A. Istituto Sperimentale Agrumicoltura**

**Presence of a new CTV focus in Calabria, Italy**

After finding la arge citrus tristeza virus outbreak in field on different citrus species in Sicily and in Apulia in 2002, the C.R.A. - Istituto Sperimentale per l'Agrumicoltura of Acireale (Italy) had carried out several surveys in several Italian regions in order to monitor the phytosanitary status of Italian citrus groves.

Surveys have shown that the virus is spreading from the first foci, particularly in Sicily in the focus found in the province of Catania (Sicily) on trees of 'Tarocco old line' sweet orange grafted on sour orange. In Sicily CTV has been shown in 'Tardivo di Ciaculli' mandarin in Villabate village located in the province of Palermo (Sicily).

Prior to November, 2005 no focus of CTV had been found in other Italian citrus growing regions other than that found in Sicily and Apulia.

However, In the month of November, 2005, during the monitoring activity in other citrus Italian regions a large focus was found in a commercial orchard of about 4 ha located in Rosarno village in the province of Reggio Calabria (Calabria). The CTV infections was detected on 'Fortune' mandarin and 'Miyagawa' satsuma grafted on sour orange. The 15 year old trees showed the typical decline symptoms and pinholing beneath the bud union line. The infection has been confirmed by DTBIA and DAS Elisa. Maria Guardo of C.R.A Istituto Sperimentale per l'Agrumicoltura of Acireale has started a molecular characterization of the CTV isolates in collaboration with Walter Davino of Dipartimento di Scienze e Tecnologia Fitosanitarie Sez. Patologia of the University of Catania.

This new finding arouses particular attention because this focus is located in a dense citrus growing area, moreover after Sicily the Calabria region is the second citrus growing region of Italy and with 40,000 ha of citrus orchards it has 22.6% of citrus growing areas.

Intensive monitoring will begin to define the situation of infection.

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## **Monica Guzman - Columbia**

**Istituto de Biotecnologia Universidad Nacional de Columbia**

This year I would like to report to IOCV members that as member of the academic and organizing committee of the 46 Meeting of the American Phytopathological Society, Carribean Division to be held at Cartagena ( Colombia) on 12 to 16 September, 2006, we have invited more than 20 international speakers. Two of the principal guest will be PEDRO MORENO and RONALD BRANSKY, and a symposium of citrus and viruses will be in the program. For more information please contact the web site [www.apsnet.org/members/div/caribbean/](http://www.apsnet.org/members/div/caribbean/)

Also, in May 1 to 6 , I invite Dr. Gerd Müller to the Universidad Nacional de Colombia and Corpoica for, two talks at the University and a practical supervision on symptoms at Corpoica

Citrus Collection. My master student Patricia Rodriguez has finished her work on Lima Tahiti strain variability with specific probes detection, work that will be presented at the APS-CD meeting in Cartagena.

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## **Peter McConnell & J. M. Moody - Jamaica**

**(Letter to Richard Lee - Feb. 7 2006)**

The stem pitting strain of the Citrus Tristeza Virus (CTV) is confirmed to be present in Jamaica. As you may already know, it is the known strain of the virus that will affect citrus plants regardless of the type of rootstock it is budded unto.

Presently in Jamaica, there is a lack of the necessary knowledge to detect the stem pitting strain of CTV in large quantities. Therefore, I have been requested by the Jamaica Citrus Protection Agency Ltd. Board of Directors, to enquire of you, if you know of any laboratory method to detect the strain in large quantities. Detecting the stem pitting strain of CTV in large quantities will help us in determining how widely spread this strain is and hence assist in the elimination process.

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## **Meisaku Koizumi - Japan**

**Letter of March 15, 2006**

Thanks for having IOCV News Letter. I am now retired from jobs but still concerned with citrus diseases as a short term advisor/consultant.

At this opportunity I would like to introduce some researchers in Japan active to study on huanglongbing disease. I am now an external reviewer of the international research project for development of effective control measures of HLB. The project has been implemented by JIRCAS (Japan International Research Center for Agricultural Science, Tsukuba, Japan) since 2004 and assumed to have 8-year term. Dr. Takeshi KANO ([k371el@affrc.go.jp](mailto:k371el@affrc.go.jp)) is the project leader and other 6 scientists including two pathologists, 2 entomologists and 2 horticulturists participate in the team. Some of the activities are conducted by collaboration with SOFRI (South Vietnam Fruit Research Institute, Mytho, Vietnam) in Vietnam. Dr. Katsumi ICHINOSE ([ichis@affrc.go.jp](mailto:ichis@affrc.go.jp)), entomologist cum sub project leader is the site coordinator at SOFRI. The project had two workshops at SOFRI in 2004 and 2005 by gathering of some specialists from Asian countries and local extension staffs, who discusses strategy to combat the disease and updated data of HLB incidence and its damage. The project emphasizes to clarify mode of HLB transmission through vector, such as seasonal population trends in psylla and viruliferous one, distance of psylla-dissemination, better application methods of insecticide for controlling psylla, easy, effective and more sensitive method to detect HLB, screening of tolerant rootstock, and cultivation practices to extend productive life-span of citrus able to bring profit to farmer even under HLB-incident condition. Development of risk assessment for HLB in each field shall be one of the outputs. Some outputs are now feed backed to local farmers to combat the disease.

Because HLB has been found in Ryukyu Islands since 1989 and moving up to Kyushu where is major to produce citrus in Japan, a development of reliable and practical detection method for HLB is urgent. Dr. Toru IWANAMI ([tiwsw37@affrc.go.jp](mailto:tiwsw37@affrc.go.jp)) is a leader to improve indexing method by PCR. In Okinawa where HLB is prevalent Dr. Shinji KAWANO ([kawanosh@pref.okinawa.jp](mailto:kawanosh@pref.okinawa.jp)), the

head of plant pathology, Okinawa Agricultural Research Center is conducting many activities to combat HLB such as identification of HLB in fields, conducting eradication program, and production and maintenance of disease-free citrus nursery stocks. Dr. Keiji YASUDA ([yasudaki@pref.okinawa.jp](mailto:yasudaki@pref.okinawa.jp)), the director of plant protection division cum head of entomology is conducting to reveal vector's behavior in field and to develop insecticide-application programs to control HLB.

Myself, M. KOIZUMI ([koizumi-m.s@tbi.t-com.ne.jp](mailto:koizumi-m.s@tbi.t-com.ne.jp)) achieved 4 field trips to survey HLB in Thailand, Vietnam and Okinawa for last 2 years. In early 2005 a field survey in northern Thailand was conducted by collaboration with Mr. Maitree Promintara, DOA-Thailand. Certification program and production of disease-free nurseries were well conducted by national research center as well as by private sector. However, a few number of plant was contaminated with HLB at the beginning of planting in field. It was obvious that citrus orchard became fully declined through vector transmission within 5 years after planting if insecticide sprays were inadequate. It was also noticed that many orchards of 10-15-years old Som Keaw Wan (local mandarin) in upland were still productive even under incidence of HLB. Some cultivation practices employed by farmer seemed effective to elongate life-span of fruit production. In January 2006 a short term study in Mekong Delta, Vietnam was conducted by collaboration of SOFRI members to identify the causal agent of citrus yellow decline, which often occur on King Mandarin on Volkameriana rootstock in Mekong region. In some cases YD symptoms occur alone, but in many cases they were accompanied with HLB symptoms because of much prevalence of HLB. Preliminary results strongly suggested that YD would be due to root-rot caused by *Phytophthora* spp. Further study to confirm the pathogen and selection of rootstock for YD are now undertaken by JIRCAS-SOFRI.

In Okinawa HLB is prevalent in Shii-kuwasha (local mandarin, *C. depressa*), particular in the trees planted in home-garden. Large numbers of commercial orchard are still free from HLB, because frequent sprays of chemical are employed. Because of good price of Shii-kuwasha most farmers have been much incentive to combat the disease, while some inhabitants are still reluctant to eradicate HLB infected trees from their back yards.

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## Hennie LeRoux - South Africa

Extension Manager Citrus Research International Ltd.  
A letter related to greening disease in South Africa - March, 2006

If we say that greening control is working in South Africa we mean that it is working for the time being. We have 24 Citrus Technology Transfer groups (study groups) in southern Africa and we are constantly reminding the growers of the danger. If the problem is neglected we shall be back to where we were within one decade. Prof Bove would not regard South Africa's case as a success story as none of the research that was conducted to learn more about Greening comes from South Africa. The increase in export cartons tells a different story.

There is also a major difference between African greening and Asian greening. Asian greening is much more aggressive than African greening. The vector of African greening has a much narrower host range and the climate is in most of the citrus producing areas only suitable for the host for a couple of months of the year. I also believe that there are more peasant farmers in China and the Philippines which make the coordinated control of the disease impossible. If the vector is controlled in South Africa for 4 - 5 months of the year the spread of the disease is controlled.

Another reason why it is easier to control the spread of the disease in South Africa is because of the Citrus Improvement Programme. At the moment 95% of all citrus trees produced within South Africa and Swaziland are produced within this scheme and we are working on legislation to clamp down on the other 5% backyard nursery trees. I am sure that in the case of the Philippines such a programme do not exist. (I may be wrong).

Lastly, I believe that in the rest of the world the citrus industries has overlooked the benefits of the stem applied systemic insecticides. The use of monocrotophos and methmidophos as stem applications are so cheap on young trees that it should be affordable up to an age of 5 - 6 years. If you give an orchard in a greening infected area that head start you have won half the battle. If not, remove your trees and buy your citrus from South Africa.

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## Juliet Ohasan - Philippines

Bureau of Plant Industry, Baguio City

I accessed the Ecoport, and I am impressed. one thing that impressed me the old pictures from Philippines, how you were able to keep those old slides, so that we can see it and use even at this time. at least I have an appreciation of the previous work done. I think even if I go to Batangas right now, nobody can show me those pictures. it is also very timely that I am preparing a lecture for the growers for next week in our farmer field school (FFS). I will give a lecture about huanglongbing as one of the component of the FFS on special topics. so I borrowed some of your slides and included it on my power point presentation. I really like to show that huanglongbing is a problem all over the citrus world and almost everywhere the management is the same - that it is important to understand the disease and implement control at critical periods.

I am really putting my effort into these small group of interested growers if only to save the industry in this part of the country and to sustain the work we started, because I am fully convinced that there's money in citrus, and the growers themselves are earning. one grower ( who is a young civil engineer by profession)invested on citrus and is about to leave for IRAQ to work because he is not confident that citrus would give him enough to feed his family, but he was convinced to stay put and concentrate on his citrus trees (only 1 hectare), after he realized a gross of almost 900,000 pesos only in the 2nd year of bearing, with only 300 full bearing trees. So he decided to stay put and is encouraging his co-growers that indeed they need not go abroad. so I am really committed to help these small group of growers, especially in the battle against HLB. Of course, there are sad stories, like one retired couple, I have to have recommend cutting most of their trees because they were severely infected. what is sad is that this couple, who were very industrious did not know that their trees were diseased, so they just keep on fertilizing and cleaning hoping it will recover. they now joined with the group of growers in the FFS, because they intend to

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## Peng Wei - China

College of Agr. Management ,Sichuan Province

Overview of the Dwarf Late-maturing Shaddock free-virus seedling research in Sichuan, P.R.C. Sichuan Agricultural Management Academy, (Chengdu, 610072, P.R.C.) In 1989, an exotic dwarf plant, which was both early harvest and late ripening, was found in thousands of Latter-white shaddock in Suining, Sichuan, P.R.C. and was named Dwarf Late-maturing Shaddock after a series of successful experiments and research. From 2003 to 2005, 17 plants of this

new type were examined in the field for horticultural properties and pathologic symptoms and were also indexed in the laboratory. Citrus tristezza virus were found in the samples by field investigation and by ELISA. *Xanthomonas campestris* pv. citri (Hasse) Dye, Citrus exocortis viroid, Citrus tatter leaf virus or Huanglongbing was found in the field testing, while *Xanthomonas campestris* pv. citri (Hasse) Dye was discovered in one plant in serum-testing, and Citrus tatter leaf virus was found in two plants. No Huanglongbing was found by PCR. Dwarf Late-maturing Shaddock is now free-virus after shoot tip grafting and Thermotherapy.

Studies on the virus-free seedlings of the Dwarf Later-maturing Shaddock of Sichuan Province in China. College of Agricultural Management of Sichuan Province (Chengdu, China 610072) From 1989 to this date, 100,000 trees of the 'Later-white Shaddocks' were planted at Suining, Sichuan province in China. One exceptionally dwarfed plant was selected which required studies and breeding. It has three favorable qualities: it is dwarfed, early maturing and some were later-maturing. From 2003 to 2005, 17 plants were investigated and indexed pathological diseases. It was indexed and we found it that they carried Citrus tristezza virus. They were not indexed for *Xanthomonas campestris* pv. citri (Hass) Dye., the Citrus exocortis viroid, the Citrus tatter leaf virus and Huanglongbing diseases. By ELISA we found one plant to have *Xanthomonas campestris* pv. citri (Hass) Dye. Two plants had Citrus tatter leaf virus, and we have not indexed for Huanglongbing.

By shoot tip grafting and heat treatment we obtained virus-free budwood of the Dwarf Later-maturing Shaddock.

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## Gerhard Pietersen,

Citrus Research International, South Africa.

Citrus research at the newly revived plant virology program at Citrus Research International, South Africa.

A proud history of citrus virus research existed in South Africa. However, over the past few decades new research posts have not been created and vacated posts often not filled, resulting in an ever-diminishing research capacity. To address this problem Citrus Research International (CRI) (Citrus Growers Association of southern Africa – including Mozambique, Swaziland and Zimbabwe) decided to revive the plant virology program at the institute by appointing Dr. Fanie van Vuuren, who had taken early retirement a few months previously, and shortly thereafter, Dr. Gerhard Pietersen, to research posts. Fanie, was a particularly valuable appointment given the wealth of experience on citrus virus and other graft-transmissible pathogens that he brings with him. Gerhard, a plant virologist with experience in a wide-range of viruses and crops, previously headed the Plant Virology Division of the South African Agricultural Research Council's Plant Protection Research Institute in Pretoria. While having no experience of citrus viruses he has extensive experience in detection, identification and control of viruses, and under Fanie's guidance is likely to gain insight into citrus virology very rapidly. While Fanie is based at the CRI headquarters in Nelspruit, Mpumalanga province, in the middle of a citrus production area, Gerhard was seconded to the University of Pretoria, some 300km's from Nelspruit, in order to have access to postgraduate students and an extensive scientific community. Fanie and Gerhard are supported by Kobus Breytenbach, a former assistant to Lawrence Marais at CRI, Katherine Stewart, a CRI prestigious bursary winner, who is undertaking an MSc. at the University of Pretoria, and Jacolene Meyer, a former assistant of Fanie, who has

a shared post with the banana tissue culture laboratories of duRoi and who will be based with Fanie and Kobus in Nelspruit.

Research at the CRI Plant Virology division is mainly centred around support of the citrus improvement program (CIP). To this end, Fanie's research involves mainly evaluation of mild strains of Citrus tristezza virus (CTV) for use as cross protecting isolates, and a search for ever milder, more efficient cross protecting mild isolates. Gerhard's task initially will be to establish virus detection and identification procedures at CRI in support of the CIP and the phytosanitary authorities. Rapid, sensitive and accurate diagnosis and identification of pathogens is fundamental to the development of any strategy for their management and control. Development of such techniques is also an essential first-step in most research actions involving such pathogens. A large number of rapid tests developed worldwide are PCR-based, are clearly described in the literature with pathogen and primer sequences deposited in Genbank or available in published articles, and therefore there is no need to re-develop detection techniques until unique South African pathogens or strains are demonstrated. The establishment of such techniques locally is thus feasible, requiring essentially just local synthesis of primers and a positive control with which to assess them. An infectious entity to serve as a positive control is not necessarily required, with either previous amplicons with the same primer set or a clone of the relevant target sequence serving to assess the ability of the technique to detect local isolates (where available or present) in plants. In this regard Gerhard has already experienced the generosity and helpfulness of the worldwide Citrus Virology community, with positive controls and guidance provided by imminent citrus scientists, including Pedro Moreno, Siddarama Gowda, Rene' Bovey, Mark Hilf, Moshe Bar-Joseph, Richard Lee, Mari Carmen Vives, Juliano Ayres, Waldir de Jesus Junior, Diva Teixeira, Elliot Kitajima, John Fisher, Colette Saillard, Takao Ito, Julania Freitas-Astua, Nuria Duran-Vila and others too numerous to mention, with thanks.

To address the control of CTV by cross protection, and to exploit the probable underlying mechanism of RNA-silencing, it is imperative that information on the variability of this virus be obtained locally. Techniques such as SSCP have previously been used by Fanie and his former group, and yielded useful information. An alternative is to obtain more sequence information to variable regions. These areas must be identified first before obtaining sequence information is generated. Micro-array analysis is ideally suited to this. Fanie, Gerhard and Katherine's second aim is to generate information on the variability of the local mild-strain cross protecting CTV populations used in South Africa using Mark Hilf's molecular markers and a supplementary micro-array analysis. Once such techniques are available the dynamics of cross protection by CTV strains will be studied. (Pietersen center & Van Vuuren Rt.)



## Marylou Polek - California

Central California Tristeza Eradication Agency

Given the severe disease situations in Florida and other locations, tristeza seems like a walk in the park. Growers in selected pest control districts continue to survey and remove trees diagnosed as infected with Citrus tristeza virus. They are very concerned about the potential (probable?) introductions of diseases currently exotic to California and remain on alert and ready to take action if necessary.

From July 2004 through October 2005, a total of 1,539 trees were diagnosed with CTV and removed. Although this number is exceptionally high, the overall disease incidence remains relatively low, 0.106%. Virus titer is just now high enough to begin the 2006 spring collection season. We will start collecting field samples the last week of March.

The Agency began using a new batch of polyclonal antibody prepared by Drs. Olga Nikolaeva and Alex Karasev. The transition occurred in the fall of 2005 and went extremely smoothly.

There are currently 361 California CTV isolates in the collection. Additional information regarding the biological reactions of these isolates can be found on the Agency's web site at [www.cctea.org](http://www.cctea.org).

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## Fanie vanVuuren - South Africa

Citrus Research International, South Africa.

Letter of 26 October, 2005

Yes, I had an accident in June when my tractor went down a steep cliff with me after the brakes failed. I fell off the tractor and went about halfway down the cliff while the tractor went down to the bottom. I broke my femur near the hip and with surgery it was fixed with a steel plate and screws. Recovery is slowly (because of my age) and I am now starting to walk without crutches when I am in the house. (March, 2006 Fanie is mostly recovered).

On the greening front we have nothing new except that we are using embryo rescue from healthy chimeras of infected fruit to obtain possible resistant/tolerant plants. For the control program it is important to start with healthy plants. Nurseries in greening areas must have protection against the vector and they are not allowed to sell plants in 'greening-free' areas. After planting in the field the trees are kept permanently poisoned with systemic insecticides for the first 3-4 years prior to bearing fruit. When the trees start bearing they are protected during the main growth periods by either insecticides aimed specifically at the vector or insecticides targeted at the control of other insects and will control the vector as well ie. citrus thrips. Scouting for the presence of the vector is very important, especially near natural bush. Infection still takes place, especially from nearby bush, and these branches should be removed.

Just to comment on the problems and failures of the greening control program in certain regions. For a number of years everything went well with the program. The weather assisted with vector numbers low. Some growers found comfort in the situation and were not so alert when high populations suddenly occurred. When they realized it, the damage was done. I visited a big estate close to Nelspruit at the time and saw that the life cycle of the vector was completed on the trees. That told me that there was no vector control.

## Le Thi Thu Hong - Viet Nam

Southern Fruit Research Institute

Viet Nam is one of hot spot counties of Citrus Huanglongbing (HLB). SOFRI (Southern Fruit Research Institute) with the supports from International projects from Australia (ACIAR) Japan (JIRCAS) France (CIRAD) Taiwan (FFTC), we have put many efforts in solving this problem. Some new findings could be brief mentioned as follows: There were two strains of Candidatus Liberibacter asiaticus in South Vietnam as a preliminary identification by PCR-RELP.

After 5 years the two demonstration blocks of Nam Roi Pummelo at Long Dinh and Thoi Son which are 30m and 500m from the HLB hot spot areas respectively, showed that Long Dinh orchard got re-infestation more than 30 % while Thoi son orchard was reinfected only 6% .

Confidor applied directly at the root base once/month with 0.15g/ plant gave good result on vector control as there was only 21.1% PCR positives and 2 % declined plants compared with Bassa with 74.2% and 60% declined plants while the controls got 95.6 % and 70% PCR positive respectively and declined two years after planting. (seedlings were initially disease free in the severe disease areas).

Among 41 cultivars screened for HLB under high inoculum pressure, Pummelos, oranges, limes lemons and mandarins showed 64,98%, 81,63%, 85,83% and 87,27% infection respectively. After two years almost all of the cultivars showed over 60% reinfected. This implied that searching for resistant/tolerant varieties is a difficult task.

Guava crops seemed to act as a repellent for the vector as initial observation showed when citrus rotated with Guava the reinfestation was reduced significantly. The investigation on its effect and mechanism, however, are being done for more precise answers.

For disease detection, LAMP (Loop-mediated isothermal amplification) is a new method and is a simple laboratory procedure. IR (Iodine reaction) on nitro-cellular membrane is a very simple kit for farmer usage. [ltthong@hcm.vnn.vn](mailto:ltthong@hcm.vnn.vn)

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## Georgios Vidalakis - Riverside, California

Director Citrus Clonal Protection Program (CCPP)

Georgios Vidalakis was appointed as the new Director of the CCPP in 2005 one year after earning his Ph.D. degree from the Department of Plant Pathology at the University of California, Riverside. During his graduate studies he worked with Professor David Gumpf and Joseph Semancik in various projects including citrus viruses and viroids. After the untimely death of Professor Gumpf in 2003, Georgios had the privilege to work with Dr. Steven Garnsey for the completion of his doctorate. Mixed viral infections and their effect to biodiagnosis of graft-transmissible diseases of citrus as well as the biological activity, molecular properties and intrapopulation structure of citrus viroids have been part of his work.

Georgios arrived in the United States in 2000 after completing his studies at the Agricultural University of Athens (AUA), Greece with concentration in Plant Protection and Environment and specialization in Plant Virology under the

guidance of the Professor Panayota-Toula Kyriakopoulou. Recently, a European Union funded project brought Georgios together with a research team of AUA and the Greek Ministry of Foods and Agricultural Development in a effort to establish a pilot certification program for citrus in Greece.

As CCPP director Georgios will focus on the optimization of the functions of the California citrus certification program, including diagnostics (bioindexing and laboratory), therapy (shoot tip grafting and thermotherapy), as well as the production and distribution of citrus propagative material (foundation blocks and variety management

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## Changyong Zhou - China

Citrus Research Institute

Chinese Academy of Agricultural Science

This middle January (2006) Dr. Ken Bevington from Dareton, Australia visited us for evaluating the effect of the former ACIAR project on CRI and Chinese Citrus Industry assigned by ACIAR. He was impressed at the improvement of CRI.

Profs. Zhao Xueyuan and Ke Chong may visit Brazil in this coming June for attending the HLB meeting there, I have also received an invitation letter from Brazil, but unlikely to attend due to time limitation.

In a letter from Mr. Francisco Llatser (Paco) of Spain, He proposes that the next meeting of the International Society of Citrus Nurserymen (ISCN) is likely to be held in Chongqing in 2008 (a week before next ISC meeting). The final decision has not yet made, it will take another half year or more.

Our facilities and research conditions have still been being improved quickly, so we shall make more contribution to the citrus industry in research area in the future.

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## Norma Costa - Argentina

INTA EEA Concordia

ALARM IN ARGENTINA BY PRESENCE OF HUANGLONGBING (HLB) IN BRAZIL

The detection of HLB in Brazil in June 2004, has created an alarm for Argentine citriculture due to the importance of its possible consequences. *Diaphorina citri*, the HLB vector, is present in Argentina, but the disease has not been detected yet. Also, in Argentina the parasitoid *Tamarixia radiata* and the alternative host *Murraya paniculata* are found. For Argentine citriculture, the presence of this insect vector is very important since only an illegal entrance of infected propagation material (budwood) or the entrance of the insect vector carrying the disease are necessary for an epidemic. Authorities of INTA, and other responsible organizations for control, (SENASA and INASE) are on the ALERT.

It is necessary for growers and nurserymen to know about the seriousness of this disease, to be able to identify the insect vector, recognize the disease and be able to diagnose the disease. A request for diagnostic equipment, materials and trained personnel to start a monitoring program with the purpose of avoiding or diminishing the risks of entrance of this disease has been forwarded to the authorities of INTA, SENASA and INASE.

INTA has suggested measures to avoid the entrance or to

attenuate the impact:

- Plant healthy plants (from the National Certification Program)

- Control the insect vector.

Knowledge about the disease and its insect vector by Argentine nurserymen and growers may prevent the entrance across the borders or to detect it sooner.

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## Loredona Barbarossa - Italy

Istituto de Virelogia Vegetale - Bari

A very sensitive and specific method for CTV detection

During spring 2002 and 2003, large-sized foci of tristeza were identified in citrus groves of the Ionian coast of Apulia (south-east Italy), established on sour orange. In view of the massive monitoring to be carried out for assessing the sanitary status of the groves in the area of the out-breaks, a nonradioactive dot-blot hybridisation assay for the detection of *Citrus tristeza virus* (CTV) RNA in total nucleic acid extracts of infected citrus was developed. Two digoxigenin(DIG)-labeled minus-sense riboprobes, complementary to the coat protein gene sequence of two different CTV isolates were synthesised. The method was tested on isolates of different geographic origins and on field-grown samples and was able to detect the virus in extracts from different citrus hosts. Because of the high sensitivity and reliability of the assay and the polyvalence of the riboprobes, the whole protocol might be easily and successfully applied as a diagnostic technique in sanitary and certification programmes.

The results of this work are described in a manuscript scheduled to appear in Journal of Phytopathology 154 (5)

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## Norma Costa -- María Inés Plata - Argentina

INTA EEA Concordia

PSOROSIS IN ARGENTINA – Natural Spread

At INTA- EEA Concordia, continuing research started by Rodriguez Pujol, Beñatena, Portillo and Danós, we are working on natural spread of psorosis (CPsV),.

A 23 yr-old plot of sweet orange seedling trees, with ten trees under screen to protect them against insects (Portillo and Beñatena, 1983, "Natural Spread of psorosis in sweet orange seedlings, Proceedings of the 9<sup>th</sup> IOCVP pp.159-164), with trees showing bark scaling and foliar symptoms was analyzed by TAS-ELISA and biological methods giving positive results for CPsV with both methods. The ten trees under screen are free of CTV and psorosis (CPsV).Data will be analyzed for a natural spread pattern. (Addendum - Pictures of these plots can be seen in the Ecoport slide show #66 showing the natural spread 3 and 11 years after the 1979 planting).

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## Michael Kesinger - Florida

Florida Citrus Budwood Protection Program

Florida citrus nurserymen were proactive in forming a



Nursery Improvement Committee over a month prior to the first find of citrus greening in Florida in late August 2006. This committee's name was changed to the Florida Citrus Plant Protection Committee (FCPPC) and has met eight times since July. The (FCPPC) is working with the Division of Plant Industry to establish new sanitation procedures for citrus nursery trees. The initial draft of a new budwood rule has been written that will require all citrus nursery and budwood production to occur in enclosed structures with double entry ways. While established nurseries are allowed to remain on their current sites, new nurseries must be started 1-mile away from any commercial citrus planting. Existing nurseries will have until 1/1/2007 to complete the enclosure requirement for producing new nursery stock. With the loss of field nursery production, tree demand is expected to exceed supply in the short term until enough greenhouse capacity is built.

Florida's foundation budwood will be isolated in new greenhouses outside of the commercial citrus growing region of the state. Plans are underway to construct a new budwood facility that will provide protection by building insect exclusion structures. Commercial citrus nurseries will have to have their budwood sources enclosed by 1/1/2007 and all budwood scion trees will have to move 1-mile away from commercial citrus groves by 2010. The new Citrus Nursery Stock Certification Program will address many other nursery sanitation issues and bring tree standards to a new level in Florida.

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### Lopes et al. 2005 - Brazil

(in Brazil, pruning has no effect on the control of of the Brazilian strain of huanglongbing)

Ineficácia da poda no controle do huanglongbing dos citros. Lopes, S.A., Frare, G.F., Yamamoto, P.T. & Ayres, A.J. Fundecitrus, C.P. 391, 14807-040, Araraquara, SP; e-mail slopes@fundecitrus.com.br. Inefficacy of pruning to control citrus huanglongbing.

*Huanglongbing* (HLB), causada por *Candidatus Liberibacter americanus* e *Ca. L. asiaticus*, foi descrita no Brasil, Estado de São Paulo, em março de 2004. Medidas de controle incluem plantio de mudas sadias, eliminação das plantas sintomáticas e controle do inseto vetor. Este estudo objetivou verificar se a poda também poderia ser usada no controle. Um total de 677 plantas de Valência (3, 5 e 16 anos) e Hamlim, Lima e Pêra (5, 7 e 9 anos) assintomáticas (nível 0), com sintomas na extremidade (nível 1) ou em toda a extensão dos ramos (nível 2) foram podadas, removendo-se somente a pernada com sintomas ou toda a copa (corte feito no tronco). Foram feitas aplicações de inseticidas em toda a área e engaioladas diversas plantas. Foram avaliadas as folhas das brotações novas e ramos remanescentes, e feitas análises por PCR. Seis meses após a poda, 7, 46 e 61% das plantas níveis 0, 1 e 2 podadas na pernada, e 8, 60 e 65% das plantas níveis 0, 1 e 2 podadas no tronco, manifestavam sintomas. Somente *Ca. L. americanus* foi detectada. A poda foi ineficaz em remover os tecidos colonizados pelo patógeno e, portanto, não deve ser usada no controle do HLB. Somente a eliminação das plantas sintomáticas reduz fontes de inóculo.

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### Gustavo Nolasco - Portugal

Universidade do Algarve, Faro

*Toxoptera citricida* on a leaf and flower in Northern Portugal

The BrCA *Toxoptera citricida* seen on a leaf and flower in northern Portugal. In a letter from Gustavo Nolasco "Last week (September 20, 2005) I have been again in the north of Portugal more or less at the same places we had been. This time I took some ELISA reagents and did some assays in place. I found some CTV infected plants infested also by *T. Citricidus*. The CTV infections were confirmed at the lab by PCR. The prospect of an CTV outbreak is now more probable."



Showing the brown citrus aphid (*Toxoptera citricida*) on a leaf and flower of citrus in northern Portugal in the Spring of 2005.

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### CTV in the top 100 of *Virology*

Erik Mirkov

In December 2005, *Virology* celebrated 50 years of publishing key research in the field of virology. In the 50<sup>th</sup> anniversary special edition of January 2006, "A Publisher's Note" by Tessa M. Picknett listed the top 100 most frequently cited articles in *Virology* to date. Of interest in this list is the fact that 5 of the top 100 papers are on plant virology research. Of more interest is that of these 5, coming in at number 67 of the top 100 most frequently cited articles in *Virology* to date is the following paper:

A. V. Karasev, V. P. Boyko, S. Gowda, O. V. Nikolaeva, M. E. Hilf, E. V. Koonin, C. L. Niblett, K. Cline, D. J. Gumpf, R. F. Lee, S. M. Garnsey, D. J. Lewandowski and W. O. Dawson. 1995 Complete sequence of the citrus tristeza virus RNA genome. *Virology* 208(2): 511-20.

Way to go "Team CTV"

Update on citrus greening (huanglongbing) in Florida

Susan Halbert and Wayne Dixon

Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

The Florida Department of Agriculture and Consumer Services (FDACS), with cooperation from the USDA, has been surveying for huanglongbing (HLB) since its discovery in the Miami area in August 2005. To date, we have found infected plants in twelve counties. The first delimiting survey indicated that infected residential citrus trees are common in the urban areas of southeast Florida

(Miami-Dade, Broward, and Palm Beach Counties). We also have found infected plants in commercial citrus groves, but so far, HLB-infected areas are limited. A combined FDACS and USDA survey of approximately half of the square mile sections with commercial citrus in Southwest Florida resulted in positive finds in several groves; however, we did not find symptomatic plants in most of the sections we surveyed. Based on the surveys, State and Federal officials have determined that HLB cannot be eradicated in Florida.

In our experience in Florida, symptoms of HLB are variable, and sometimes ephemeral. Plants with classic blotchy mottle may decline to the point where mottled leaves are gone, and the few remaining leaves are yellow. Similarly, known infected plants also may appear to recover during the spring flush. Occasionally, plants with apparent classic mottle symptoms are PCR-negative. We have a lot to learn about HLB, particularly field symptomatology and laboratory diagnostics.

A Citrus Health Response Plan (CHRP) to deal with HLB, canker, and other pests and diseases of citrus is under development by FDACS and USDA. The CHRP will provide science-based best management practices (BMPs) for dealing with HLB and canker.

One of the difficulties we have encountered in development of BMPs for HLB is that research on the disease organism is restricted due to its status as a "select agent" under the Agricultural Bioterrorism Protection Act of 2002. Select agent status is assigned to those pathogens that are thought to have potential use as biological weapons. Once HLB is delisted, research will move ahead on culturing the HLB bacteria and conducting insect transmission experiments, which are essential priority topics.

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## NEWS FROM TEXAS

*John da Graça*

The discovery of Huanglongbing (greening) in Florida has caused some concern in Texas, where *Diaphorina citri* was first recorded in 2001. A survey will be conducted by the Texas A & M University-Kingsville Citrus Center and USDA-APHIS-PPQ, with collaboration from Texas Dept. of Agriculture, Texas Cooperative Extension and others, to determine how widespread the psyllid vector and its predators and parasites have become, and if any trees are infected with HLB. The commercial citrus industry is concentrated in the Lower Rio Grande Valley (LRGV) in south Texas next to the border of north east Mexico. However, small plantings of citrus as well as many garden citrus trees are grown in many other parts of the state. In particular, there are many trees in the Houston area, and in areas between Houston and the border with Louisiana to the east. Isolated citrus can also be found in other locations scattered around the state; one with historical significance is Carrizo Springs approx. 300 km NW of the LRGV, which gave its name to the Carrizo citrange and where a few trees are grown.

Some of the research that has been done by virologists here (with some collaboration with Florida and Spain) on *Citrus tristeza virus* here has been published in the recent past:

Herron, C.M., T.E. Mirkov, N. Solis-Gracia, C.J. Kahlke, M. Skaria & J.V. da Graça. 2005. Severity of *Citrus tristeza virus* isolates in Texas. *Plant Disease* 89: 575-580.

Herron, C.M., T.E. Mirkov, J.V. da Graça, & R.F. Lee. 2006. *Citrus tristeza virus* transmission by the *Toxoptera citricida* vector: In vitro acquisition and transmission and infectivity immunoneutralization experiments. *Journal of Virological Methods*: doi:10.1016/j.jviromet.2006.01.006

Hernández-Jasso, J.C., J.V. da Graça & E.S. Louzada. 2004. Identification of differentially expressed genes in *Poncirus trifoliata* by *Citrus tristeza virus*

inoculation. *Subtropical Plant Science* 56: 26-32.

Vives, M.C., L. Rubio, A. Sambade, T.E. Mirkov, P. Moreno, & J. Guerri. 2005. Evidence of multiple recombination events between two RNA sequence variants with a *Citrus tristeza virus* isolate. *Virology* 331: 232-237.

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## Beatriz Stein-Tucumán, Argentina

Citrus Sanitation Center of the Estación Experimental Agroindustrial Obispo Colombes, (EEAOC)

After one year and a half of its establishment 104 plants of lemons, oranges, and citrus hybrids had been obtained by shoot tip grafting at the Citrus Sanitation Center. The biological indexing for Citrus tristeza virus (CTV), psorosis, exocortis and cachexia detection are in progress. CTV diagnosis was also done by immunoprint-ELISA.

Rootstock seed source trees which are used by commercial citrus nurseries are biologically indexed for detection of the Citrus psorosis virus. These seed source trees must be psorosis free in order to be part of the certification program.

In August, 2005 Dr. Roistacher visited our facilities to advise on our indexing operation and certification program and in November, 2005 Beatriz Stein visited the University of California Riverside. Dr. Roistacher was an excellent host and with his kind guidance the facilities, research work and researcher of the Citrus Clonal Protection Program, the National Clonal Germplasm Repository for Citrus and Dates, the Variety collection and the Citrus breeding program were visited.

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## Chet Roistacher - California

University of California, Riverside

Roistacher continues to travel and enjoy seeing new places and meeting new friends. He was invited to Tucuman, Argentina in August, 2005 to review their certification program and was deeply impressed with their facilities and their desire to learn. He was also impressed with their wonderful staff under the leadership of Beatriz Stein who replaced the former citrus director Jose Luis Foguet. I had a chance to meet Dr. Fuguet who is now consulting. Of interest was the finding of a naturally transmitted psorosis spreading in a sweet orange grove. (see Ecoport slide show No. 66 and also the newsletter input on natural spread of psorosis by Norma Costa Pg. 8.)

In September, I attended a workshop in Portugal by FAO-CIHEAM on "A Harmonized Programme for the control of CTV and *Toxoptera citricida* in the Mediterranean" (see Nolasco Pg. 9). In September, 2005 I spoke at the meeting of the International Society of Citrus Nurserymen in Cairo, Egypt. My presentations at both workshops in Portugal and Egypt was: "The Threat of Citrus Tristeza Virus to the countries of the Mediterranean Region". Anna Maria D'Onghia and I had a chance to discuss this situation with the Minister of Agriculture and present the serious potential threat to Egypt by both tristeza and greening disease. Again, in January, along with Anna Maria D'Onghia we finalized the GTZ project in Egypt and spoke with the new Minister of Agriculture in on these two serious threats (Fig 1).

In March, 2006 I taught my 20<sup>th</sup> year in Italy at the Istituto Agronomico Mediterraneo di Bari and was given a surprise party at which the entire staff presented me with an amazing poster of an orange tree with fruit showing each of my 20 classes from 1986 to 2006. I was deeply touched by this honor (Fig. 2).

## Matilde Tessitori – Catania, Italy

### CTV (*Citrus tristeza virus*) in Sicily

After the records of different foci of CTV in Italy the Department of Phytosanitary Sciences and Technologies is leading a survey to collect data in order to depict a preliminary representation of the CTV epidemic in Sicily. The Plant pathology Laboratory of the Science and Technology Park (managed by A. Catara) appointed by the regional service is also active in the program as well as the Institute of Citriculture (ISAG) and the Regional Phytosanitary Service.

An extensive survey in the Catania Province, that has 40% of citrus orchards of the region (corresponding to more than 40.000 ha), has revealed a variable incidence of CTV according to the peculiar orography of citriculture (from coastal to mountain areas) and number of varieties.

Results of evaluation of about 5000 DAS-ELISA tests (by using the mixture of monoclonal antibodies) is complex but, as expected, depicts a different situation in the various areas and varieties. The virus has been detected in a high percentage in two main areas of the Province while it was sporadically present or absent in other important citrus areas and varieties. The vector *Toxoptera citricidus* appears to be still absent in our region.

The survey is going on with more analyses according to hierarchical sampling method. These first results address future criteria of monitoring activity in the peculiar situation of the region, in order to establish the urgent control measures.

Further researches on the local effectiveness in CTV transmission of *Aphis gossypii* (or other vectors) and genomic characterization of virus isolates detected will give tools for the forecast of CTV epidemic in our conditions.

AVAILABLE PROCEEDINGS OF THE IOCV Federal ID No. (FIDN) 51 014 8324

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8	30.00	5.00	35.00	15.00	45.00
9	30.00	5.00	35.00	20.00	50.00
10	30.00	5.00	35.00	20.00	50.00
11	35.00	5.00	40.00	20.00	55.00
12	35.00	5.00	40.00	20.00	55.00
13	40.00	5.00	45.00	20.00	60.00
14	40.00	5.00	45.00	20.00	60.00
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(15<sup>th</sup> Proceedings to be available shortly)

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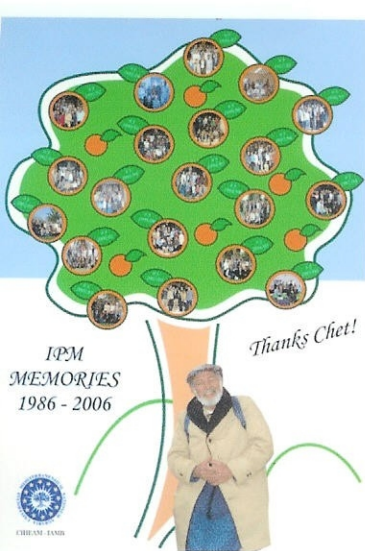
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Send to:

Chester N. Roistacher, Secretary/Treasurer, IOCV  
 Dept. Plant Pathology, University of California  
 Riverside, CA 92521-0122 USA

NOTE TO MEMBERS - E-MAIL ADDRESS UPDATE – PLEASE SEND YOUR UPDATED E-MAIL ADDRESS

TO C. N. ROISTACHER - [chetroist@charter.net](mailto:chetroist@charter.net) Fax: (951) 827-4398



Left: Poster honoring 20 years teaching at IAM-Bari  
 Above: Meeting with the Minister of Agriculture in Egypt, January, 2006  
 Below: New indexing facilities Tucuman, Argentina

Above: Bulldozing greening trees in Brazil



Left: Successful control of greening disease in South Africa. This area was once devastated with greening disease.

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