INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

IOCV NEWSLETTER **October, 2006**

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From the Chairman

In just 12 months time, we will be gathering in Adana, Turkey, for the 17th IOCV conference, and our 50th birthday. Please take note of the news elsewhere in this newsletter of a slight change in the dates. We will be working with Nukut Onelge and her committee to make the conference a success (as IOCV meetings always are), but the most important ingredients are the participants, so please start making your plans to attend now.

Here we are planning the 17th conference, and you are wondering "Where are the Proceedings of the 16th?" All contributors recently received galley proofs, and Mark Hilf reports that almost everyone responded promptly. Invoices were sent out at the same time, so hopefully you can arrange payment – IOCV is a non-profit organization, and does not have large reserves in the bank, so we need the page charge payments in order to pay the printers. Your cooperation will be appreciated. If you have problems in transferring funds, or need to arrange paying in installments, please contact the Treasurer, Robert Krueger.

Once the proceedings have been distributed, we will begin the selection process for the judging of papers for Wallace and Gumpf awards. The winners will be announced in Turkey.

Looking ahead, we have also initiated discussions on possible venues for the 18th conference in 2010. I appointed a committee composed of the Board of Directors and former Chairmen to look at some possibilities. I now invite all IOCV members to begin discussing possibilities. We will need to have proposed venues presented at the meetings in Adana, with sufficient details of possible conference site, approximate dates, and associated tours to enable members to make a wise choice. Three possible venues have been mentioned to me, and if they develop into real possibilities, they will be presented there, but you may have an even better proposal.

Here in the United States many of us are focusing on Huanglongbing. Reports of new finds continue to come out of Florida, and surveys in other states to determine the presence/absence of *Diaphorina citri* and HLB are moving forward. Thus far, the disease has only been found in Florida, while the vector (so far without HLB) is widespread in Texas, and has been reported in Hawaii. No psyllids have been found in California, Arizona, Louisiana or Alabama.

Several IOCV members attended the International HLB Symposium in the Brazilian city of Riberão Preto in July – it resembled a mini-IOCV conference. Juliano Ayres and his fellow committee members arranged an excellent meeting. I think many of us found the presentations by Brazilian regulatory officials and growers, and the field trip, particularly beneficial.

John da Graca

From Nuket Onelge
Change of 17th IOCV Conference dates

We have changed the date of IOCV Conference in Adana, Turkey because there is a conflict with our religious festival. The new conference dates will be October 18-21/2007 for the pre-conference (in Adana city); October 22-26/2007 for the conference (in Adana city) and October 27-30/2007 for the post conference (in Antalya city). The Conference program will be announced at this website in October: http://iocv2007.cu.edu.tr.

News from Texas
John da Graca

Following the discovery of Huanglongbing in Florida in 2005, other citrus producing states in the US became very concerned, especially Texas where the Asian citrus psyllid has been present since 2001. USDA-APHIS-PPQ is funding surveys in Texas, California, Arizona and Louisiana to determine whether the vector is present, and if so how widespread it has become, and if any HLB can be detected. In Texas, John da Graca and Mani Skaria report that they, along with entomologists at the Citrus Center (Victor French, Mamoudou Setamou and Patrick Haslem), have been surveying both the commercial citrus areas in the Lower Rio Grande Valley, as well as other areas where citrus is grown in small orchards and as trees in backyards. Psyllids have been found in many areas throughout south Texas, and in some areas further north. Some were found in the large cities of San Antonio and Houston, but none in the area of east Texas adjacent to Louisiana. No typical HLB symptoms have been observed, but many leaves with deficiency-like symptoms have been tested by PCR, and all have been negative.

A graduate student at the Citrus Center, Tina Thomas, recently completed her master's degree in Mani's lab. She studied the reported tolerance/resistance of viroid-infected citrus to *Phytophthora*, originally described in Brazil by Victoria Rossetti, and more recently a subject Joe Semancik reported on. Tina observed that viroid-infected citrus roots produced contained differing levels of phenolic compounds, and that the fungus developed very few sporangia on infected tissue compared to non viroid-infected tissues. Analysis of the viroid sources showed that they contained more than one species, but so far no correlation between resistance to Phytophthora and a particular viroid has been found. In addition to exocortis, cachexia and *Citrus viroid III*, one source was found to contain CVd-IV, a viroid previously found in only seven other states or countries.

Erik Mirkov continues his journey towards disease resistance through transgenics. One promising development from his lab, although not of a viral nature, a Rio Red grapefruit transgenic for the spinach defensin gene has shown a good level of resistance to canker in tests performed in Florida. The inoculum level was very high, yet there is a marked visual difference (see photo at the end of newsletter).

Tristeza Survey, Italy
Mario Davino & Angelo Caruso

Citrus Tristeza Virus **was detected in open field for the first time in Calabria in 1982 (Davino et al., 1983) on material imported illegally from other countries. Subsequently, thousands imported ornamental citrus plants were found to be infected in several nurseries in Tuscany (Davino and Terranova, 1999). In the last years the situation has dramatically changed. In fact, thousands infected trees of various citrus cultivars have been found in commercial citrus orchards in Sicily, Calabria and Apulia (Davino et al., 2003). In the last four years both the Department of Phytosanitary Sciences and Technologies, University of Catania and the "Istituto Sperimentale per l'Agrumicoltura" in collaboration, have tested more than 50,000 citrus trees of various cultivars (see Table). All CTV isolates recovered from infected trees have been characterized (Davino et al., 2003a, b; Davino et al, 2004; Davino et al., 2005a,b) Researches of commercial citrus orchards in Sicily revealed that 90% of aphid populations was constituted by *Aphis gossypii,* while *A. fabae* and *Macrosiphon euphorbiae* accounted for 5% each (Davino et al., 2004). Since 1985 it was demonstrated that aphid species in Sicily are able to transmit CTV isolates (Davino & Patti, 1985; Davino et al., 1989). Thus far *Toxoptera citricida* has not been found in citrus orchards in Sicily. In a citrus growing area of Eastern Sicily it was observed that the proportion of CTV infected trees increased from 21 to 90% within four years. The data suggests that CTV has been present for many years in commercial citrus orchards in Southern Italy.**

Gottwald Honored
Edward B. Knipling, ARS Administrator

Tim Gottwald, research leader was honored at the Florida Fruit and Vegetable Association. On September 19, Calvin Arnold, director, ARS U.S. Horticultural Research Laboratory, Ft. Pierce, FL, and Timothy Gottwald, research leader of the lab's Subtropical Plant Pathology Research Unit, attended the 2006 Florida Fruit & Vegetable Association Convention in Orlando, FL. Gottwald was honored as the association's 2006 Researcher of the Year, in recognition of his significant research achievements benefiting Florida's fruit, vegetable, tropical fruit, and sugar cane industries. Specifically cited was his 2002 paper:

Citrus Canker: The Pathogen and Its Impact Gottwald, T. R., Graham, J. H., and Schubert, T. S.12 August 2002.

Summary: The U.S. has seen an increase in introductions of invasive plant pests into agricultural crops. In Florida, one such invasive species is a bacterial plant pathogen that causes Asiatic citrus canker. Its eradication has received considerable press attention and legal challenges, has produced far-reaching political and socioeconomic impact in Florida, and has implications for national and international trade.

Irey et al. 2006

Post-hurricane Analysis of Citrus Canker Spread and Progress towards the Development of a Predictive Model to Estimate Disease Spread Due to Catastrophic Weather Events M. Irey, T. R. Gottwald, J. H. Graham, T. D. Riley, and G. Carlton. August 2006.

Summary: Geospatially referenced citrus canker infection data from infections that were discovered after the 2004 hurricane season were examined in relation to wind and rain conditions experienced during the hurricanes and used to develop a predictive model to explain storm-related spread of citrus canker. The results suggest that the predictive model shows great promise a tool to predict disease spread as a result of extreme weather events and as a means of targeting resources for citrus canker survey and detection activities.

News from Fort Pierce, Florida
Mark Hilf, USDA-ARS

This summer in Fort Pierce was rather quiet, with no major hurricanes to contend with this year. Bob Owens, USDA-ARS, Beltsville Maryland visited the USDA station in Fort Pierce Sept. 17-19, to evaluate the progress of a field plot to study viroid dwarfing in grapefruit that is part of a collaborative project with Mark Hilf and Kim Bowman of the Plant Pathology and Horticulture and Breeding units at the USDA in Fort Pierce. Toru Iwanami, Chief Scientist of the Citrus Greening Research Team at the National Institute of Fruit Tree Science, Tsukuba, Japan, will visit Florida from Oct. 31-Nov.9. Toru will visit with Bill Dawson at the Univ. of Florida, Lake Alfred and with Tim Gottwald and Mark Hilf at the USDA to discuss and present research on citrus greening and to see Florida greening symptoms in the field. From Dec. 5-7, Tim Gottwald will attend and present at an International Workshop for the "Prevention of Citrus Greening Disease in Severely Infected Areas", which will be held in Ishigaki, Okinawa, Japan. Tim will join with other international experts to discuss what is an ongoing disease problem in many citrus areas, but which is a new and growing problem in Florida.

Marina Barba - Italy

Virus and virus-like diseases department of CRA-Istituto Sperimentale per la Patologia Vegetale, Roma, routinely investigates the sanitary status of citrus plants in central Italy for the presence of Citrus Tristeza Virus (CTV) by means of collaboration with regional phytosanitary service of Latium. These analyses are carried out with serological (ELISA) and/or molecular tests (RT-PCR).

Moreover, in the frame of the national Project "Citrus fruit growing" supported by Italian Ministry of Agriculture, researches on citrus viroids started in our Institute in 2003.

A one-tube one-step RT-PCR was developed and utilized for detection of CEVd and HSVd in citrus field-plants collected in several citrus orchards of the main citrus growing areas and nurseries of Latium and Tuscany regions (Ragozzino et al., 2004). This technique proved to be very reliable, sensitive, easier and more rapid compared with other protocols actually in use for citrus viroids diagnosis. The results of our investigation showed the presence of CEVd and HSVd in the , respectively, 100% and 30% of the samples from Latium region and 45% and 9% from Tuscany region.

Recently, the Institute has been invited by local service (Calabria region) to investigate on the sanitary status of citrus plants grown in Calabria, a region of South Italy very important for the cultivation of this crop. This collaboration will cover a period of 3 years for the phytosanitary control of citrus plants either in nursery production or in the field. Mother plants will be investigated for the presence of the most important viroids and viruses, with particular attention to CTV.

Up to now, about 500 samples of different Citrus species, mainly Lemon and Orange, collected from Calabria nurseries, have been processed in order to detect, by ELISA (and/or by molecular methods, if necessary), the presence of CTV. At the moment all samples resulted negative for this virus. We analyzed also about 50 mother plants for the presence of *Citrus Psorosis Virus* (CPsV) and, at the moment, it seems that the disease, and the associated virus, is not present in the inspected nurseries.

Publications of interest to IOCV membership:

Ragozzino E., Faggioli F., Barba M., 2004. Development of a one tube-one step RT-PCR protocol for the detection of seven viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid and Pospiviroid. Journal of Virological Methods, 121:25-29.

Ragozzino E., Faggioli F., Barba M, Detection and Distribution of Citrus exocortis viroid and Hop Stunt Viroid in citrus orchards of central Italy as revealed by one-step RT-PCR. 16th IOCV Conference 2005, pp. 463-465 (in press).

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Juliet Ochasan

Philippines

*** On October 16-20th I will be conducting a training session on disease indexing on citrus and banana here in my laboratory in Baguio City, using ELISA and PCR. About 25 are expected to attend from all over the country.**

*** On October 24-26th we have a national fruit symposium, where I will be presenting a paper on the production of quality plant materials of citrus.**

Here are some developments on the citrus industry and upcoming events:

***The issuance by the Secretary of Agriculture of Administrative Order #6 series of 2006, which provides the guidelines on the production, regulation, promotion, procurement and distribution of seeds and planting materials. This includes citrus plant material certification and accreditation of citrus nurseries. This is a welcome development as it has been several decades of attempting to put in place a certification system for citrus.**

*** Fifteen growers graduated from the first Farmer Field School (FFS) on citrus, which was piloted from March 2005 to May 2006, in one of the Cordillera provinces. I was one of the three facilitators.**

*** Upcoming events: October 9-13 - I was requested by the local government unit of Kasibu, Nueva Viscaya to lead a task force on citrus Huanglongbing eradication. This will be the first attempt to eradicate trees showing typical Huanglongbing symptoms. This is an initiative of the grower's cooperative and the local government.**

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Xylella fastidiosa in citrus orchards of the State of São Paulo, Brazil

Helvécio D.Coletta Filho, Kely C. S. Alves, Hélio R. Triboni & Marcos A Machado

Citrus Variegated Chlorosis (CVC) still remains a serious disease in sweet orange orchards of the State of São Paulo, Brazil. The disease incidence across the citrus growing regions of São Paulo is known in detail from Fundecitrus surveys (www.fundecitrus.com.br/english/est_cvc_us.html), but the knowledge about the bacterium incidence is still not evident. In 2005, almost one thousand samples from Northern (435), Central (410) and Southern (326) citrus growing regions of São Paulo State were collected and analyzed by PCR for X. fastidiosa and visually for CVC symptoms. Plants with different ages (0 – 2, 3 – 5, 6 -10, and > 10 years old), within each region, were sampled. The frequency of X. fastidiosa and disease symptoms are shown in the table bellow. In the Northern and Central regions, the incidence of X. fastidiosa was higher, as initially expected since the incidence of the disease in both regions were also higher. In the Southern region, in spite of the low disease incidence, a relatively high frequency of the bacterium was observed. Potentially associated with this result is the longer drought period, during the winter, observed mainly in both Northern and Central regions then in Southern São Paulo areas. As already pointed by previous work, the expression of CVC symptoms may have a strong environmental influence, potentially associated to water stress. Plants until two years old showed lower incidence of both the disease and the bacterium, emphasizing the importance of the certificated nursery programs, which has been mandatory since 2002, and the initial field management of CVC for controlling the disease.

Percentage of incidence of Citrus Variegated Chlorosis (CVC) and Xylella fastidiosa (X.f.) in orchards from São Paulo State, according with age of plants and geographic regions.

Age (Yrs)	CVC[1]	X.f.[2]	Geographic regions	CVC	X.f.
0 - 2	2	4	North	67	74
3 - 5	21	23	Central	48	52
6 – 10	34	57	South	5	35
> 10	50	65	Average	41	59

1Data from all regions of Sao Paulo State.

2Based on the percentage of positive PCR.

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Arguments for a plant laboratory in any comprehensive indexing program.

C. N. Roistacher

Mention is sometimes made to do away with the plant index in a certification program since there are now many excellent laboratory techniques for the detection of graft-transmissible pathogens of citrus. It is my belief that plants are still not only needed, but are a necessity in any comprehensive indexing program.

The California Certification program and indexing. In developing the California Certification Program back in the early 1950's, I had the responsibility to develop an indexing program for citrus for the Variety Improvement Program, later called the California Citrus Clonal Protection Program. (EcoPort slide program ID 164)

http://ecoport.org/ep?SearchType=slideshowView&slideshowId=164

This was a grave responsibility, for if I should miss detection of any virus or viroid or pathogen, hundreds of thousands of buds would be distributed throughout the State of California and since our budwood was exported to countries worldwide, this missed pathogen would be present and it would have my name on it. Therefore great care and research was done to develop the UC system of plant growth for citrus so that the finest plants would be grown to enable detection of the mildest reacting pathogens. (See the UC system of plant growth - EcoPort slide show ID 82)

http://ecoport.org/ep?SearchType=slideshowView&slideshowId=82

Bio-multiplication. Bio-multiplication is the unique property of plants to multiply pathogens from a very few to many millions when a plant is cut back after inoculation. When virus or other pathogen titer is low they can be missed by molecular detection methods, plants will rarely if ever miss the presence of the pathogen after inoculation. In comparative tests, ELISA failed to detect the Citrus tatter leaf virus (in Texas and in Thailand) when temperatures were too warm whereas plant indexing was uniformly successful. Similarly, it has been shown that ELISA will fail to detect the Citrus tristeza virus and the Citrus psorosis virus during the warm summer months.

Pathogens detected only in plants. (Others can be found by reviewing the IOCV proceedings)

Concave gum (Worldwide)

Impietratura (Europe, South Africa and North Africa)

Cristacortis. (Europe and North Africa)

Severe strains of Citrus tristeza virus such as the Peru, Capao Bonito or 12B isolates.

Fatal yellows (California)

Yellow vein (California)

Various Yellow vein diseases in Pakistan and India

Gummy bark (in all countries of the Middle East)

Citrus measles (Florida & Brazil)

Rubbery wood (India)

In addition, there are a number of citrus pathogens for which specific laboratory detection procedures have been developed and these individual assays would have to be included in any comprehensive non-biological indexing program. However, in a comprehensive indexing program where a large range of index plants are used, the probability for detection of these pathogens - or new pathogens, would be materially increased.

The plant can pick up certain dwarfing factors where no virus or pathogen is present (Roistacher et al. 1972 - p. 199-204. In Proc. 5th Conf. IOCV, Univ. Fla. Press, Gainesville.).

In summary, I believe that the plant is still a necessity in any comprehensive indexing program.

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Reunion Islands

Christian Vernier

(In response to an E-mail on the disappearance of the vectors of HLB in Reunion)

I have been working on citrus canker for 18 months in Reunion island in wonderful new facilities located in the south of the island in Saint-Pierre(beautiful structure with very good and complete equipement for plant pathologists (from gene to epidemiology).

Huanglongbing disease in Réunion is under control for years. Even citrus producers forgot about it!

After the introduction of *Tamarixia dryi* in 1974, specific parasite of *Trioza erytreae*, this African psyllid disappeared after 5 years. Actually, a second host of *T. dryi* existed in Reunion which was the psyllid of the giant bramble (*Rubus alccifolius*, a wild invasive species), named *Trioza litseae* or *T. eastopi*, which was present near the orchards. *Tamarixia dryi* was then always present in this host close to the citrus. When the African psyllid came in the orchard, it was attacked by the parasite. As *T. dryi* preferred *T. eryrtreae*, this psyllid was destroyed. *T. eryrtreae* was not observed anymore in Reunion for 25 years. Normally, it is not the case and an equilibrium takes place between the parasite and the host, but because of the presence of an alternative host, the "preferred" host disappeared.

A specific parasite of the Asian psyllid (*Diaphorina citri*) was also introduced in 1978. This parasite *Tamarixia radiata* established an equilibrium with the Asian psyllid. This psyllid was not present anymore in the citrus orchards but persisted in populations more or less important in the hedges of *Murraya paniculata*. Recently, one technician tried to capture some Asian psyllids for one of our researcher working on HLB (Frédéric Gatineau) and it was very difficult to find some.

So we cannot say that we have completely eradicated HLB, and some Liberibactors could be present in some trees. But, for many years, there was no complaining about declining citrus in the orchards due to HLB.

Citrus in Reunion without HLB (greening disease)

A new view of the Parent Washington navel orange tree

C. N. Roistacher

The mother of all Washington navel orange trees resides in Riverside, California and is now 133 years old. When it was 100 years old, it was indexed and two pathogens were found: Vein enation virus and Citrus viroid IIa. Budwood from the parent tree was shoot tip grafted and these pathogens were subsequently eliminated. Two trees of the three trees which were originally brought to California by rail in 1873 survived. One tree was trampled by a cow shortly after planting. A second tree was planted at the historical Mission Inn in Riverside in 1903 by the then President Theodore Roosevelt. However, this tree died of Phytophthora root rot. The third tree was removed to a dedicated fenced park at the corner of Magnolia and Arlington Avenues and cared for by the Riverside Parks Department. However, since this tree was on sweet orange rootstock, it began to decline with Phytophthora about 1915 but was save by inarching in 1918 and was inarched again in 1951.

In September, 2006 a meeting, called by the Riverside Parks Department to assess the needs of this mother tree to assure its continuing health and survival. During this meeting, which was held at its fenced Memorial Park, we were allowed inside the fence to study the tree at close range. This was a new experience for me to be able to study the large growing inarches. However, at close range I could see for the first time the original sweet orange stump showing severe lesions of Phytophthora gummosis which could not be previously be seen from behind the fence. Shown in the photographs below is a view of the Parent navel tree taken in 2005 and a close up view of the grown inarches and the Phytophthora lesions on the original sweet orange trunk which almost destroyed this historical tree.

For a complete picture essay on the History of the parent Washington navel orange, visit the EcoPort website and view slide show #79. This link will take you directly to this slide show:

http://ecoport.org/ep?SearchType=slideshowView&slideshowId=79

BELOW – THE PARENT NAVEL ORANGE TREE IN 2005

Magally Williams - CDFA - California

The California Department of Food and Agriculture (CDFA) started, this spring, the Statewide Huanglongbing Survey as a part of the USDA National Huanglongbing Survey.

The survey targeted citrus and citrus relatives looking for symptoms of the disease and the disease vector, the Asian citrus psyllid (*Diaphorina citri*), in residential areas, ornamental nurseries that carry citrus and/or *Murraya paniculata* (Orange Jessamine). Surveys in commercial citrus groves targeted the Asian Citrus Psyllid.

The purpose of the survey is to detect the presence of Huanglongbing and its vector in California before either has a chance to become established in the state. These are the results:

Properties surveyed	**4648**
Nurseries surveyed	**270**
Samples taken	**264**
Positive found	**0**

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Florida Science Source

Will Wordowsky of the Florida Science Source has, over the many years, been most helpful in distributing our IOCV proceedings. He has a new published second edition of "Fresh Citrus Fruits", A description is in the attached file.
http://www.ultimatecitrus.com/fssource/index.html

I asked Will if he had any copies of Volumes 1 through 6 of the IOCV proceedings which are currently out of print and otherwise not available and he replied that he had six copies of Volume 2 for $25.00 each and nine copies of Volume 4 for $33.00 each.

He can be contacted by e-mail at: fssource@aol.com

ON RIGHT – SHOWING THE ORIGINAL SWEET ORANGE TRUNK (IN CENTER) WITH LESIONS OF PHYTOPHTHORA

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Detection of tristeza in Calabria, Italy

Giuliana Albanese and Vincenzo Palmeri
Universitàà Mediterranea di Reggio Calabria-Italys

After the recent detection of hundred 'Fortune' mandarin and satsuma trees affected by citrus tristeza virus (CTV) in the Region of Calabria (South-Italy) (Caruso *et al.*, Informatore Fitopatologico 2006, 5-6), the local administration has financed a 3-year (2006-2009) program aimed at improving screening, prevention and eradication of CTV in this area. In order to identify location and level of CTV infestation in citrus nurseries and commercial plantings, a survey, conducted by the Department of "Gestione dei Sistemi Agrari e Forestali" (GESAF), Università *Mediterranea* di Reggio Calabria, in collaboration with CRA-Istituto Sperimentale per la Patologia Vegetale, Roma, has been set up since June, 2006.

Up to this date, about 2,000 samples, each composed by five different trees, were harvested in the four main citrus-growing areas of Calabria. Samples were assayed for presence of CTV by DAS-ELISA. All samples from citrus nurseries were CTV-free. Among commercial plantings, only 18 samples of 'Moro', 'Navelina', 'Ovale', 'Washington Navel' sweet orange, and satsuma, all grafted on sour orange and collected in citrus orchards of the Reggio Calabria Province, were positive. Of note, these CTV-infections were found in the same area where a previous infection *focus* had been described thus, suggesting aphid transmission of the virus in the field. By contrast, no infection was found in the other four Calabrian Provinces.

In the meanwhile, a survey on CTV-vectors in Calabrian citrus-growing is in progress to identify citrus aphid species and to characterize the pathosystem of CTV spread. *Aphis gossypii, Myzus persicae* and *Toxoptera aurantii* were the most common species detected; as expected, *Toxoptera citricida* was not found.

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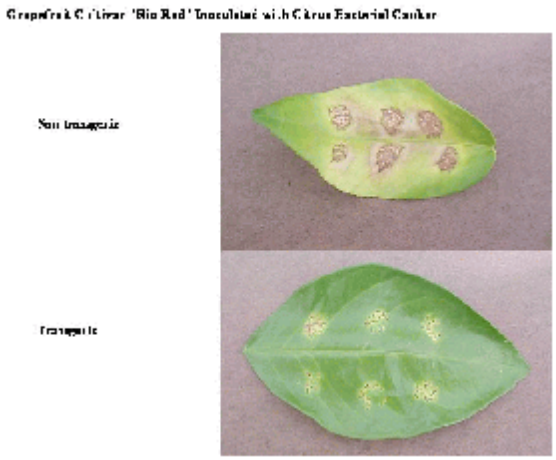
Below - transgenic resistance to Citrus canker - Mirkov



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INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

MEMBERSHIP APPLICATION IN IOCV

The International Organization of Citrus Virologists (IOCV) is an independent, non-profit association for the promotion of excellence and advancement of research with virus and virus-like diseases of citrus. Membership is open to anyone who is interested in the exchange of information on diseases of citrus.

A membership fee of $30.00 US, payable to IOCV is required for the period between the 15th and 16th conference of the IOCV (for the three year period 2004-2007) . Student fee is $15.00.

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