

INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

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IOCV NEWSLETTER

June, 2000

FROM THE CHAIRMAN

Dear Friends,

As you can see from the following information supplied by Anastasia Kyriakou from Cyprus and Moshe Bar-Joseph for the next IOCV meeting in Cyprus (with a post conference trip) and for a pre-conference tour of Israel and Jordan, are well under way. We are grateful for the time and effort they are putting into the preparations.

I hope I may catch up with some of you at the ISC meetings in Florida in December. If any of you are coming to the Olympic games in Sydney, Australia in September this year, please make contact with me by e-mail at < or by telephone (61) 2 46 406450 (as I live and work not far from Sydney)

Finally, I would like to thank everyone who has contributed to the success of this newsletter.

With best wishes,
Pat (Broadbent) Barkley

15TH CONFERENCE IOCV - CYPRUS

Anastasia Kyriakou

A draft of the First Announcement for the XV Conference of the IOCV was sent to Prof. Bar-Joseph for addition of the information regarding the Pre-Conference in Israel, so that we may finally have a single announcement for the Pre and the Conference. Here below follows the basic information included in the first announcement with regard to the Conference in Cyprus.

Conference Location and Programme: The registration of the delegates and Conference formal sessions will take place in the "Amathus" Hotel, Paphos, from November 11 to 16. A one-day field trip to citrus government nurseries and Commercial plantations will be encompassed. Paphos, a historic city of touristic importance today, is situated on the western coast of Cyprus in the centre of an agricultural area.

Accommodations: Special rates have been negotiated for accommodating the Conference participants in the "Amathus Beach Hotel" of Paphos, a five-star luxury hotel. Approximate daily charges are US \$80.00 for a single room and US \$66.00 per person for a double room. Efforts will be made to enable accommodation of interested participants in a more economical hotel within walking distance from "Amathus".

Two-day tour: A two-day field trip will be organized on November 17-18 to Nicosia in the center of the island and to Arnaca, at the southern coast. Visits will include places of historical and cultural interest, the Agricultural Research Institute, and some old citrus plantings.

15TH IOCV

PRE CONFERENCE TRIP - ISRAEL AND JORDAN

M. Bar-Joseph

Here is some information regarding the planned IOCV pre-conference tour in Israel and-Jordan - November 3-10, 2001

Organizing committee:

Mr. Tal Amit, CMBI

Mr. A. Sadowsky, Department of Citriculture Ministry of Agriculture (Extension)

Prof. M. Bar-Joseph, ARO, Volcani Center (Chairman)

Mr. Y Kolodner, Pelach

Mr. R. Sterenlicht, Ministry of Agriculture

We aim to show the visitors the main virus and viroid diseases affecting production, blended with tours to famous archaeological and historical sites and Institutes of Research and Higher Education.

Day 1 (Saturday) 3/11/01 Arrival and transfer to Hotels
Day 2 (Sunday) 4/11/01 Citrus in the Coastal Plain plus visit to the Volcani Center
Day 3 (Monday) 5/11/01 Citrus in the South + Massada
Day 4 (Tuesday) 6/11/01 Citrus in the North + Sea of Galilee
Day 5 (Wednesday) 7/11/01 Jordan North
Day 6 (Thursday) 8/11/01 Jordan South and Petra
Day 7 (Friday) 9/11/01 Eilat
Day 8 (Saturday) 10/11/01 Jerusalem & Flight to Cyprus

This program is still tentative. The estimated cost is about 120 US\$/day. Negotiations are in progress with a number of Companies to get the best and most economical deal.



WORKSHOP ON HUANGLONGBING - VIETNAM

Pat Barkley

A workshop on Huanglongbing (greening) was held in Hanoi, Vietnam from March 31 - April 1, sponsored by the Australian Centre for Agricultural Research (ACIAR) and CIRAD, to determine research and development priorities and possible areas of co-operation between the two aid organizations in South East Asia. Those attending from Australia were Pat Barkley, Richard Davis (plant pathologist with the Northern Australian Quarantine Organization), Andrew Beattie (citrus entomologist, University of Western Sydney), Debbie Rae (located in Guangdong, China), Paul de Barro (entomologist, CSIRO, Brisbane) and four administrators from ACIAR. Representing France were Josy Bové, Monique Garnier, Philippe Cao-Van (horticulturist located at Long Dinh in South Vietnam), Serge Quilici (entomologist from Reunion), and three CIRAD officials. Lise Korsten spoke of greening in South Africa. Siti Subandiyah from Indonesia, Ratana Sdoodee from Thailand, Nguyen Minh Chau, Huynh Tri Duc, Le Thi Thu Hong Ngo Vinh Vien and Nong Van Hai from Vietnam represented South East Asia. Areas of mutual interest to ACIAR and CIRAD were explored and researchers are now drawing up research proposals.

TOXOPTERA CITRICIDA IN MEXICO

J.P. Michaud and R. Alvarez

The brown citrus aphid (BCA), *Toxoptera citricida*, primary vector of citrus tristeza virus, has been present in Belize since 1997. During the first week of April, 2000 we surveyed commercial and dooryard citrus in central regions of the State of Yucatan, Mexico from Merida south to Oxkutzcab and Tzucucab close to the border with Quintana Roo without detecting BCA. We then extended our search eastward into the northern portions of the State of Quintana Roo, where the aphid was rumored to be present. Our first positive detection occurred at km 255 on highway 30 in the town of Santo Domingo, about 30 miles west of Cancun. A single roadside tree was observed with heavy growth of sooty-mold on some branches. Closer examination revealed large numbers of aphid cadavers adhering to terminal twigs. Several live BCA colonies were subsequently discovered some 40 meters distant, on a flushing mandarin orange tree. Specimens were sent to Dr. S. Halbert of Department of Plant Industry, Gainesville, FL for confirmation. We then proceeded northward to inspect some small commercial and subsistence citrus groves around Kantunilkin, but did not find any evidence of recent aphid activity. However, it should be noted that the entire region was nearing the end of a dry season, with very little new growth on citrus trees available for aphid colonization. We did find evidence of some previously heavy aphid populations and some live aphids on dooryard trees in the town of Leona Vicario, also on highway 30. These are the first confirmed collections of BCA in Mexico.

CALIFORNIA SUPREME COURT UPHOLDS REMOVAL ORDER FOR TRISTEZA-INFECTED TREES

MaryLou Polek,

Central California Tristeza Eradication Agency

'Court declines to hear appellants' case against lower court decisions' -- By denying a petition to review an appellate court's decision, the Supreme Court of California effectively closed the case against parties resisting the state-mandated law requiring the removal of citrus trees infected with citrus tristeza virus (CTV) in Kern County. "This is a great news," said Vic Corkins, chairman of the Central California Tristeza Eradication Agency. "We always knew we were well within the law in surveying and removing infected trees to stop the spread of this serious disease, but now it has been confirmed. The legal appeals process has been exhausted. We can now get on with tree removal." The CCTEA, Agricultural Commissioners in Kern, Fresno and Tulare Counties and the California Department of Food and Agriculture are responsible for enforcing State laws against CTV. Since 1947, citrus tristeza virus has been ranked by the state as a Class A pest. Presence of CTV in certain districts sets in motion quarantine and eradication protocol established by CDFA. For more than three years, several Kern County producers have been battling the tree removal law in court. On April 12 the California Supreme Court in a public statement denied their petition to review the Fifth Appellate District Court's decision in favor of the Agency regarding the removal of CTV-infected trees. The Appellate Court had earlier upheld a decision made by the Superior Court of Kern County that stipulated the citrus tristeza virus constitutes a public nuisance and that, by law, such trees must be removed.

Diaphorina citri IN FLORIDA

Susan Halbert

Diaphorina citri Kuwayama, the vector of citrus greening pathogens, is well-established in Florida. When *D. citri* was discovered in Florida, its distribution extended along the east coast from southern Martin County to central Broward County. We suspect that the original introduction occurred in central Palm Beach County in early 1998 or late 1997. Further natural spread over the next year extended the range of the Asian citrus psyllid from the Florida Keys (Monroe County) to southern Indian River County, and west to Clewiston (south shore of Lake Okeechobee, Hendry County).

At the time this is being written, naturally occurring infestations have been found in nine southern counties. Infestations have been found in retail stores in 11 additional counties, mostly in central Florida. Most of the finds in retail outlets have been on *Murraya paniculata* plants that were propagated in south Florida. It is not known whether *D. citri* has become established in the environment in the additional 11 counties where we have records only from retail stores. Further, it is not known how far north *D. citri* is able to overwinter in Florida.

It appears that natural colonization by *D. citri* of newly invaded areas occurs at approximately the same rate as colonization by exotic aphids, about 250 km per year (Wellings 1994). That rate of colonization was observed for *Toxoptera citricida* (Kirkaldy) when it colonized the Florida citrus belt in 1995-1997. Unlike *T. citricida*, *D. citri* has moved extensively on plants for sale, particularly *M. paniculata*. There are several reasons for this. First, *T. citricida* primarily colonizes citrus, and many of the major citrus nurseries in Florida are located in some of the later areas of the Florida citrus belt to be colonized by the aphid. Second, commercial citrus nurseries are aware of the serious nature of *T. citricida* infestations, so insecticide treatment is common; whereas *M. paniculata* is a landscape ornamental that is not likely to be treated for a pest that doesn't drastically reduce salability of the plants. Finally, *T. citricida* is a more conspicuous pest than *D. citri*. *Diaphorina citri* eggs, in particular, may be very difficult to see even at high density.

So far, *D. citri* has not proven to be a major pest in Florida citrus. We do not expect it to become a major pest unless the citrus greening pathogens are also introduced into Florida. We have done extensive visual surveys and processed a limited number of PCR samples to look for citrus greening. So far, we have not found any evidence of citrus greening disease in Florida.

Literature Cited:

Wellings, P.W. 1994.

How variable are rates of colonization? *European Journal of Entomology* 91: 121-125.

MONIQUE GARNIER - FRANCE

Stubborn/*Spiroplasma citri*

Three papers concerning the characterization of a non pathogenic mutant of *S.citri* (mutant GMT 553) are *in press*. They show that fructose utilization by the spiroplasma is linked to pathogenicity, probably by impairing sucrose loading into the phloem sieve tubes by the companion cells.

Gaurivaud, P., Laigret, F., Garnier, M., & Bové, J.M. 2000. Fructose utilization and pathogenicity of *Spiroplasma citri* : characterization of the fructose operon. *Gene In press*.

Gaurivaud, P., Laigret, F., Garnier, M., and Bové, J.M. 2000. *Spiroplasma citri* : isolation and characterization of

fructose operon mutants. *Microbiology. In press.*

Gaurivaud, P., Danet, J.L., Laigret, F., Garnier, M., and Bové, J.M. 2000. Fructose utilization and pathogenicity of *Spiroplasma citri*. *Molecular Plant Microb Interact. In press*

A non motile mutant of *S.citri* (mutant scm1) has been shown to be able to cross both the gut epithelium and the salivary gland membrane. Thus it is not affected in its transmissibility and pathogenicity.

Duret S., Danet J.L., M. Garnier and J. Renaudin, 1999. Gene disruption through homologous recombination in *Spiroplasma citri*: an scm1 disrupted motility mutant is pathogenic. *J. Bacteriology* 181: 7449-7456.

A transposition mutant of *S.citri* (G76), able to multiply to high titer in the vector *Circulifer haematoceps*, has been selected because it was transmitted to only 50% of periwinkle plants when the wild type spiroplasma was transmitted to 100% of the plants. In addition, it induces late symptoms in the plant compared to the wild type, but this seems to be linked to the presence of fewer spiroplasmas in the plant. The transposon interrupted gene code for a putative lipoprotein with appreciable homology with lipoproteins of *Mycoplasma genitalium* and *M. pneumoniae*. Further studies are underway to understand the role of this lipoprotein in insects and plants.

Comparison of protein patterns of vector-transmissible and non transmissible strains of *S.citri*, have shown that two proteins of 160kDa and 60kDa were present on the PAGE profiles of the non transmissible strains only. Purification of these proteins is underway.

Huanglongbing/ *Liberobacter*

A *Liberobacter* subspecies has been discovered and characterized in an ornamental rutaceous tree Cape Chestnut (*Calodendrum capense*) in South Africa. Description of this new subspecies is *in press*

Garnier M., S. Jagoueix-Eveillard, P. Cronje, H. Le Roux, and J. M. Bové (2000). Genomic characterization of a liberobacter present in an ornamental Rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. Proposal for a 'Candidatus *Liberobacter africanus* subspecies capensis'. *International J. of Systematic and Environmental Microbiology. In press.*

In this paper we also explained why «*Liberobacter africanum*» is changed to «*Liberobacter africanus*» and «*Liberobacter asiaticum*» to «*Liberobacter asiaticus*».

CVC/ *Xylella fastidiosa*

Patricia Brant Monteiro from Fundecitrus/FAPESP, (Brazil) has been in a post doctoral position in the laboratory since November, 1998. During her stay, she developed conditions for the inoculation of periwinkle plants for the multiplication of *X. fastidiosa*, to rapidly screen putative pathogenesis mutants. In parallel, she worked on genetic transformation of *Xylella*. She will go back to Brazil on June 2 hopefully with positive results.

MONICA GUZMAN- COLOMBIA

Here is a summary of our CTV activities in Colombia. Last year I obtained my PhD at the Universite de Bordeaux, France with Dr. J.M. Bové, as my thesis director in biological, serological, and molecular characterization of CTV isolates in Corsica. After my return from France in December, 1998. I am currently collaborating with J. Penaranda and O. Acosta's CTV group. I am looking for stem pitting sweet orange strains detected by serology. There are three students working with Colombian CTV isolates. Oscar Oliveros a Ph.D candidate who is working with p27 gene variability by nucleotide sequencing and SSCP variants from six field clones isolates. Yaneth Torres, a master student, who is working with HSP65 gene sequence from two isolates and polyclonal antibodies detection. Also Gonzalo Morales a master student who is looking for CTV diversity by serology, dsRNA, enzyme restriction profiles and SSCP (CP-PCR) in a group of field samples from Antioquia region.

ISCN NEWS

(From ICNS newsletter - Charles Youtsey)

The proceedings of the 5th Congress of the International Society of Citrus Nurserymen, held in Montpellier, France, March 5-8, 1997, have been published and are available through La Librairie du Cirad BP 5035, 34032, Montpellier Cedex I, France. Fax 33(0)467615547 <librairie@cirad.fr>. Two volumes are published in the refereed journal FRUITS. Volume 52 contains issues of citrus diseases transmitted by graft or vector or by natural spread. Volume 53 contains issues of genetics. The third volume contains papers given in the seven formal sessions, i.e.: Nursery Management and Planting Techniques, Certification Schemes, Biotechnology and Germplasm management, control of Pests and Diseases, a satellite meeting in Morocco, Citrus Genetics and Biotechnology, and Graft Transmissible Diseases. Copies of ISCN Proceedings from all previous meetings are still available at nominal cost, plus mailing expense. Contact Dr. Bob Rouse, 2686 St. Rd. 29 north, Immokalee, FL

34142-9515. Fax 941-658-3469, e-mail <rer@icon.imok.ufl.edu>.

BERNARD AUBERT - FRANCE

Aubert reports his travel to IVIA Valencia where a meeting was held on Mediterranean citrus. Francisco Llatser advised that Spanish nurseries have recently boomed to 7 million citrus plants. Bernard traveled to Haiti to help with the establishment of a citrus nursery program there. He met Rolland Cottin in Corsica (SRA Corsica) and was informed of the indorsement by IPGRI-FAO of the new Citrus Descriptor. This new book should be in circulation early in 2000, and will be the official system for describing citrus varieties/cultivars. Bernard is also re-editing 'THE NATURAL HISTORY OF CITRUS', written in 1818-1822. Volume 1 of the book of 500 pages will have 110 pages of full page color plates of citrus specimens updated by modern techniques. Volume 2 will contain historical, scientific and artistic background followed by an overview of modern and old Mediterranean germplasm of the 1810's. A second part will present the ever challenging problems of graft-transmissible diseases by J.M. Bove. Pre-printing prices are \$250 US. Final cost \$334 US. Contact CME-11,rue Tronchet-75008 Paris, FR. Fax 01-42-66-13-07.

FIELD NURSERIES BANNED IN SAO PAULO

Jose DeLima (From the ISCN newsletter)

Citrus field nurseries in Sao Paulo, the largest citrus planting in the world, are numbered. Field nurseries have been banned by law beginning in January 2001. The restrictions apply to commercial, as well as own-use nurseries. The main objective is to provide Citrus Variegated Chlorosis (CVC)-free plants to the citrus industry. CVC is a systemic disease caused by xylem-inhabiting bacteria (*Xylella fastidiosa*). Only trees produced in containers or plastic bags, under screen protection, will be allowed. The measure is controversial among citrus growers and nurserymen, some citrus consultants and technical experts, but strongly supported by many industry experts, scientists, agricultural officials and most influential industry organizations. Protected plants are 50 to 100% more expensive. CVC only affects oranges among the commercial varieties. Tangerines, limes, lemons and other varieties are not affected, but growers will have to pay more for those varieties as well. Some orange varieties are also showing good tolerance to CVC. In addition, the disease is widespread in the citrus areas and significant portions of the industry show milder behavior to the disease compared to others. Should CVC become a severe disease in all citrus areas, and resistant clones prove hard to come by, disease free, 10 month old plants may emerge as a true solution to the problem, and the harsh measures hereon adopted will certainly be worth the effort and cost which the industry is willing to pay now. Dr. Ary Salibe reports that there are about 1000 open field nurseries (17,639,867 trees) now in Brazil, plus 140 protected nurseries to be planted in 1999/2000 season. Rootstocks are: Rangpur 83%, Cleo 10.5%, Swingle 3.18%, Trifoliolate 2.17% plus miscellaneous.

ICS 2000

You are reminded of the Ninth Congress of the International Society of Citriculture, which will take place in December 3rd through the 7th of this year in Orlando, Florida at Walt Disney World's Coronado Springs Resort. Contact ISC, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850 USA e-mail <isc-convener@lal.ufl.edu>

CITRUS CANKER-FLORIDA

(From the ISCN newsletter - Thanks to Charles Youtsey)

Eradication efforts for bacterial citrus canker have been in effect in Florida since the 1980's. The disease has spread through the movement of plants, equipment, and strong winds and rain from three hurricanes and numerous summer thunder-storms. Over 1000 square miles from Palm Beach County to Homestead have been quarantined for the movement of citrus plants and products. Over 260 square miles in four other counties from Tampa bay to Immokalee on the west coast are similarly quarantined. 1.5 million trees are projected to be destroyed in the mostly residential areas of the east coast. All trees within 1900 feet of an infected tree are considered exposed and are being removed. Over 200,000 trees have been removed in the east coast area, including 40% of the commercial lime plantings in the Homestead area. 175 million dollars has been appropriated to the eradication program and a 40 million dollar fund for compensation payments has been established. It is hoped that within one year most of the infected trees will have been destroyed. Citrus nurserymen are required to implement strict sanitary procedures with their workers and protect their stock from plant and equipment movement. Should a citrus nursery fall within a quarantine area, all movement of plants will be halted until the quarantine is lifted, (which may be as long as two years) rendering the young trees unsalable. Compensation has been proposed for nurseries finding themselves in such a situation. So far this has not been a reality.

A. M. D'ONGHIA & KHALED DJELOUAH - ITALY

In our laboratory, we are carrying on the research on psorosis by serology, on sanitation by somatic embryogenesis from style culture and on the assessment of the sanitary status of citrus in the Mediterranean. We have demonstrated that the citrus psorosis virus (CPsV) is not seed-transmissible and that it is completely eliminated by somatic embryogenesis from the style. Moreover, immunoprinting for CPsV detection is giving very interesting results, which will soon be published. Concerning sanitation by somatic embryogenesis, we already have some results on plants infected by other virus and virus-like agents (beside CPsV and viroids), in particular for CTV, CVV, concave gum, cristicortis and impietratura. These results are about to be published. Two surveys were conducted in Malta, and in Albania for the virus and virus-like diseases of citrus. Tristeza was not found in Malta despite good conditions for the virus, while in Albania, CPsV was not detected in any of the trees sampled. Several trees, showing symptoms of woody gall, are being tested for the detection of CVEV. The GTZ project on 'Improvements of the citrus production in Egypt' goes well after one year and there are definitive improvements. Selection of citrus candidate stocks and monitoring of the most important pathogens are two activities carried out through a MSc thesis at this Institute, by Hesham Fahmy, who is in charge of the project. Besides these main research lines we are also characterizing two nepoviruses isolated from lemon which may be associated with the crinkly leaf type of symptoms.

D.K. GHOSH - INDIA

My Institute, the National Research Centre for Citrus (NRCC), in Nagpur, has been included under the World Bank funded National Agricultural Technology Project (NATP) on development of plant virus diagnostics. Prof. Y. S. Ahlawat is the National leader in-charge. Presently I am engaged in biological, serological and molecular characterization of different CTV isolates, with the objective of identifying stable mild strain for cross protection and implementing a citrus budwood certification programme to provide disease-free planting material to farmers. Recently I have identified field grown Nagpur mandarin plants in central India with phytoplasmal witches' broom, one branch showing typical broom like symptoms as was earlier noticed in acid limes.

IQRAR KHAN - OMAN

Regarding Witches' broom disease of limes, I wish to submit that we have started a multi-disciplinary program which is just one year old. It includes changing propagation from air-layering (marcotting) to one based on rootstocks; developing clean stocks; symptom repression studies; nutrient status (95% of 330 trees surveyed had zinc deficiency and N and Mn problems). Also, correctional work has begun to harvest fruit from sick trees; molecular biology to develop PCR based diagnostic probes; the identification of a vector for witches' broom; somatic hybridization for developing resistant scions. FAO has sanctioned a Technical Cooperation Project for the ministry and we hope to interact with the FAO/ministry. I am sure IOCV is aware that the disease has been reported from Iran and India. I have now submitted a proposal to FAO/IAEA for a joint research program for initiation of a germplasm collection, enhancement and evaluation study.

LEANDRO PENYA - SPAIN

My lab is actively working with citrus transgenic plants as a tool for investigating pathogenicity determinants of citrus tristeza virus (CTV) as well as obtaining resistance against this important disease. We are getting very exciting results and currently submitting them for publication.

MEISAKU KOIZUMI - JAPAN

I have just returned from visiting some research institutes in Mainland China. In April, 1999, I resigned from the position of Department of Citriculture, NIFT, Japan, and assumed the Assistant Director General of the Asian Vegetable Research and Development Center, a Taiwan based International Research Center. My target crops changed from citrus to vegetables, but I am still concerned with research activities in citrus, including virology and breeding for disease resistance. Sumiko and I quite enjoy our new circumstances. My friends in Taiwan and Mainland China sometimes invite us to visit citrus orchards and asked for my technical assistance for citrus research and disease control. Last week I visited the Citrus Research Institute, Chinese Academy of Agricultural Science, Chongqing and exchanged technical information with its staff. Control of Huanglongbing (greening) in Taiwan is being achieved by planting pathogen-free nurseries, effective timing sprays of chemicals and appropriate cultural practices. Tetracycline injections to Buntan (Shaddock) trees have done very well, due to improving the ingredients of solution and proper timing and quantity. Symptomatic trees were annually injected with chemicals and thus far, some phytotoxic and disease symptoms still remained in the trees. However, these trees are yielding normal fruit the next year after treatment. Sweet orange and mandarin trees were not subject to the injection because of mechanical damage by making holes in the trunk. We enjoy very delicious Buntan fruit

from the end of August to November in Taiwan. I would like to still be concerned with citrus virus and virus-like diseases and appreciate your contact through the following e-mail <koizumi@netra.avrdc.org.tw> (Office) or <koizm@ms33.hinet.net> (residence).

MANI SKARIA - TEXAS

Here is a briefing on a couple of activities in Texas. Caroline Herron, who is working on her Ph.D Program, is now in Florida with Chuck Niblett and Richard Lee doing some molecular characterization of some CTV isolates from Texas. Hongqin Miao, a visiting Scientist from Hebei, China joined Skaria in May, 2000. Her primary responsibility is to work on a citrus canker project. However, while collecting samples for canker, she will also be testing several dooryard citrus samples for CTV.

CRAIG J. KAHLKE - TEXAS

The Texas Citrus Budwood Certification Program has begun the sale of virus-free budwood. The bulk of the foundation block containing the main commercial cultivars that were shoot-tip grafted and indexed in Texas were planted in 1998. Non-commercial cultivars were imported from the California Citrus Certification Program and also planted in the foundation block. An increase block has been set up that is on-line to supply the entire Texas Citrus Industry with sufficient budwood of the main commercial cultivars sometime in 2001. As sufficient budwood of each cultivar becomes available, the Texas Department of Agriculture will make it mandatory to plant only virus-free trees using budwood supplied by the Citrus Center. Both virus-free blocks are located at the Texas A&M University-Kingsville Citrus Center in Weslaco.

Extensive Surveys for CTV by ELISA have revealed under a 1% infection rate in the Lower Rio Grande Valley of Texas, the main commercial citrus-growing area in the State. However, CTV surveys along the Gulf Coast of Texas, which contains mostly satsuma and other cold hardy varieties, have revealed over a 20% infection rate. No CTV disease symptoms have been seen in either area. However, citrus on the Gulf Coast is grafted onto CTV-tolerant rootstocks. A few Texas CTV isolates have tested positive against the MCA-13 monoclonal antibody as well. Well over 95% of commercial citrus is still grown on sour orange rootstock, as rootstock trials thus far have yet to find anything that has proven to work well on the variety of soil types found here. Some citrumelo hybrids are looking good, however, it is too early in the fruit evaluation period for more than limited trial plantings. With the brown citrus aphid moving quickly in Florida and Mexico, Texas is scrambling for funding to build screenhouses to protect source trees. We currently have a small 24' by 30' screenhouse that can protect only a limited supply of budwood.

T. MIYAKAWA - JAPAN

I have retired from the College of Horticulture by March, 2000. My present official address is: T. Miyakawa, Professor (emeritus), Minami Kyushu University, Takanahe, Miyazaki, 884-0003, JAPAN. My E-mail address is: <miyakawa@nankyudai.ac.jp>

Recent publication:

Miyakawa, T., Naito, T., and Ozaki, K. 2000.

Symptom expression and development of huanglongbing (citrus greening disease) under the natural conditions of southern Kyushu, Japan. p.13-26. Bull. Fac. Hort. Minami Kyushu Univ. No.30(A) (6 color fig. Japanese with English summary). (A copy of the paper is now available for distribution.)

JOHN DA GRACA - TEXAS

In April, John da Graca paid a visit to Mario Rocha-Pena in Monterrey, Mexico. Since it is only a four hour car ride, they are planning some cooperative work on CTV and viroids. The Citrus Center in Weslaco has two visitors. Miao Hongqing from Hebei, China is here for a 5-month visit, working with Mani Skaria on the CTV and canker survey. Also, Celeste Clark is here from Natal University, South Africa and has come to Texas for two months to work with John da Graca and Erik Mirkov on citrus vein enation characterization. Caroline Herron, who is doing her PhD in Weslaco on characterizing CTV strains in Texas with Erik Mirkov, Mani Skaria and myself, spent most of June in Florida with Chuck Niblett and Richard Lee using some of their probes on Texas isolates.

We, the editors for the 14th Proceedings apologize for the delay, but that we hope the quality of the Proceedings will have been worth the wait.

RICHARD LEE - FLORIDA

Dr. Padma Ramachandran is conducting research on citrus viroids with Richard Lee, at CREC, Lake

Alfred. Also, Peggy Sieburth, Florida Budwood Registration Program, Winter Haven will be in Florida on this project from May 1 through July. Her fellowship is sponsored by the Food and Agricultural Organization (FAO) of the United Nations, Rome.

STEVE GARNSEY RETIRES

Steve Garnsey, former chairman of IOCV and eminent Plant Pathologist, formally retired from the USDA at the end of 1999. The combined citrus industry of Florida (Florida Citrus Mutual, Florida Citrus Nurserymen's Association and Florida Citrus Research Foundation) combined to honor and pay tribute to Steve at a luncheon last September. We hope that Steve will continue to work with IOCV and give of his lifelong expertise in citrus pathology and virology.



ECOPORT SLIDE SHOWS

C. N. Roistacher

IOCV membership should be aware that there is a Website 2000 readily accessible which contains a large amount of visual information pertinent to citrus virus and virus-like diseases. This is the EcoPort - a data base, not just a website, which is designed and devoted entirely to inter-disciplinary integration of information to manage biodiversity. This system, sponsored by the Food and Agricultural Organization of the United Nations (FAO), the University of Florida and the Smithsonian Institute is a most exciting and informative concept, easy to access and explore and which contains a world of information. To access go to: <www.ecoport.org> click on 'resources' then click on 'slide shows'.

CURRENT SLIDE SHOWS PERTAINING TO CITRUS FOUND ON ECOPORT.ORG/RESOURCES/SLIDE SHOWS:

Lecture Number	Slide show
9	Citrus variegated chlorosis
14	Citrus tristeza virus
20	Citrus cristicortis disease
36	Witches' broom disease of limes
38	Citrus chlorotic dwarf
42	Thermotherapy of citrus
43	Evolution of pathogens
44	Gummy bark disease of sweet orange
47	Why mandatory certification
52	Med fly
55	Citrus impietratura disease
57	Concave gum disease of citrus
58	Gum pocket of trifoliolate rootstock
60	Vein enation disease of citrus
61	Satsuma dwarf disease of citrus
64	Citrus leprosis disease
65	Psorosis I - History and field symptoms
66	Psorosis II - Diagnostics and control
68	Citrus infectious variegation disease
70	Abnormal bud union of sweet/rough lemon
72	Tatter leaf disease of citrus
73	Inoculation techniques for detection of citrus pathogens
75	Citrus exocortis viroid

- 76 Citrus cachexia viroid
- 77 Wood pocket disease of lime and lemon
- 79 A history of the parent Washington navel orange
- 82 The UC system of soils and plant growth
- 85 A comic book brochure on greening disease
- 87 Stubborn disease of citrus
- 88 The death of lime trees in the Maldive Islands (Canker)

We urge members to review these slide shows for accuracy and to make suggestions, corrections and additions of slides or text to improve and enhance the slide shows. If you have a slide or suggestion please contact C. Roistacher at E-mail: <chester.r@worldnet.att.net>. All suggestions will be acknowledged and appreciated.

FANIE VAN VUUREN - SOUTH AFRICA

Fanie van Vuuren, Michael Luttig and Jacolene van der Vyver are attempt to unravel the problem of variable tristeza stem pitting development in budwood source trees pre-immunized with GFMS 12 (Nartia, B7) as well as in young commercial plantings propagated from these sources. CTV strains in South Africa are numerous and they usually occur as mixtures in natural hosts. GFMS 12 was collected in 1973 from a 47-year-old Marsh grapefruit tree (still existing and now 74 years old). It is growing on the Nartia farm near Wellington in the Western Cape Province (not a good grapefruit area). Since then, the source budline has been kept in a glasshouse and never exposed to reintroduction of additional strains by aphids. To establish the strain composition of the GFMS 12 population, single aphids were used to separate possible strains within the population. The sub-isolates that were obtained differed biologically (current IOCV proceedings). At least one of these sub-isolates appeared to be severe and induced severe stem pitting in indicator plants. Analysis of the RT-PCR products by SSCP did not show clear variation in the CP, p23 and p27 genes. Recently we found differences among these sub-isolates by analyzing a small fragment of the 3' end of the p27 gene. Currently our research involves the use of the small fragment gene to show variation of the strains in individual buds. We suspect that the variability of stem pitting in young plantings originates in the variability of the strain complex in individual buds and separation takes place during budding in the nursery.

The other two CTV populations that are used for pre-immunizing our bud wood sources, GFMS 35 and LMS 6, each consists of more than one strain but at present there is no evidence for the presence of severe strains in either population.

Currently we are harvesting field trials where we evaluate new CTV populations for their cross protection abilities in Red grapefruit selections, navel and Valencia sweet oranges and Clementine. So far, nothing better than the populations we are using has been found.

RECENT PUBLICATIONS AND REPORTS

Van der Vyver, J.B. 1999.

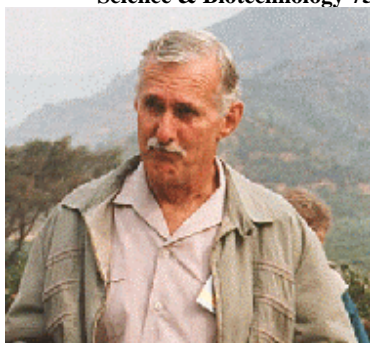
Determination of the influence of grapefruit hosts on the composition of cross-protecting citrus tristeza closterovirus populations. Research Report, University of Natal, Pietermaritzburg, South Africa.

Van Vuuren, S.P. and da Graça, J.V. 2000.

Evaluation of graft-transmissible isolates from dwarfed citrus trees as dwarfing agents. *Plant Dis.* 84: 239-242.

Van Vuuren, S.P. and da Graça, J.V. 2000.

Reduction in 'Marsh' grapefruit tree size infected with citrus tristeza virus populations. *J. of Hort. Science & Biotechnology* 75 (4): in press.



PADMA RAMACHANDRAN - INDIA/FLORIDA

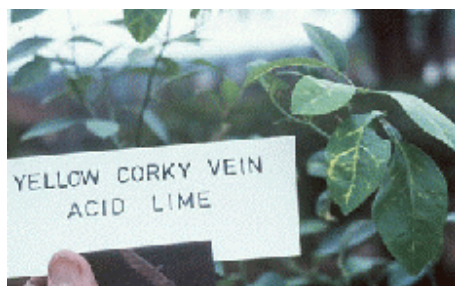
Citrus yellow corky vein – a viroid disease from India.

P. Ramachandran, F.A. Rustemand Y.S.Ahlawat, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India and Faculty of Agriculture, University of Aden, Yemen.

Citrus yellow corky vein disease is becoming important in Kagzi lime (*Citrus aurantifolia*) a commercially important variety in India. It was initially observed on sweet orange from the Southern State of Andhra Pradesh and on Kagzi lime from the Western State of Maharashtra. The causal agent was sap and graft transmissible and assumed to be caused by a virus. The disease remained insignificant until Kagzi lime orchards were found to be affected by yellow corky vein leading to nearly 90% yield loss, from Eastern State of Assam. The disease is characterized by yellow spots on leaf lamina, which soon spread along mid and lateral veins. The veins turn rough on the underside and develop corky symptoms. The affected trees showed retarded growth and bore small and hard fruits without any symptoms.

Investigations conducted at the Plant Pathology Division of Indian Agricultural Research Institute, New Delhi showed the causal agent of the disease to be viroid. Presence of a low molecular weight RNA similar in electrophoretic mobility to viroid RNA was detected in total nucleic acid extracts from symptomatic leaves, which also reacted positively to cRNA probes to citrus exocortis and hop stunt viroids in dot blot hybridization suggesting the possibility of involvement of one or more viroids with the disease. Recent studies on host range have shown that the causal viroid is experimentally transmitted to 19 hosts of citrus family (Rutaceae); Kagzi lime being a good indicator host with symptoms appearing within nine days of inoculation in hot weather. The hosts include varieties of citranges, citron, *Citrus decumana*, mandarins, lemon, lime, nasnaran, rough lemon, sour orange and sweet orange. On Etrog citron symptoms of leaf epinasty, typical of citrus exocortis, did not develop but typical yellowing appears about two months following inoculation.

In India Kagzi lime is a very popular and important variety, and the widespread occurrence of a viroid disease is threatening its cultivation. It is felt that there is an urgent need to characterize the causal viroid and develop diagnostic reagents that will prove useful in budwood certification program now being launched in the country.



Showing yellow veins (above) and yellow spots on leaves of Kagzi lime infected with citrus yellow corky vein disease. This is now shown to be caused by a viroid.

CELSO MENDES - PORTUGAL

Diagnostic of virus and virus-like diseases of Citrus and Certification Programme in Algarve, Portugal.

The Centro de Citricultura (CdC) in Faro, Portugal consists of the 'Direcção Regional de Agricultura do Algarve' (DRAALG) and Nurserymen. Since 1991, their principal objective has been the production of true to type and virus free citrus plants. For this purpose we began a collaboration between CIRAD and the CdC. We acquired 32 varieties at the San Giuliano Station-Corsica (CIRAD-IRFA). This permitted us to begin a certification programme in Portugal. We also began a foundation and multiplication block. Although the certification legislation had not been published yet, this material began to be distributed among the nurseries members. Thus, the CdC has had a fundamental role in diffusion of good quality citrus propagation material. At present the foundation block is being periodically indexed according to certification standards. We index for the psorosis complex, concave gum, cristicortis, impietratura, infectious variegation, exocortis and cachexia, all on appropriated indicators.

The DRAALG laboratories are also able to use routine methods namely ELISA and immuno-printing. Other methods like sPAGE for viroids, and PCR are being implemented. The CdC and their scientific partners

namely 'Universidade do Algarve. Campus de Gambelas, Faro' (UAlg) and the 'Instituto Nacional de Investigaçao Agrária (INIA), Lisboa are developing several national projects on citriculture, principally vegetative material production and sanitary surveys. An example is the project "New Approach for Diagnosis and Prevention of Tristeza Outbreaks", funded by a Commom Fund for Commodities, with participation of some countries of the Mediterranean region.

Another related project is a germplasm collection of some 226 different varieties that were collected throughout the country. We hold one plant in the screenhouse and two plants of each variety in the field. Some of these varieties were indexed and two mandarins cultivars ("Setubalense" and "Carvalho") were tested as virus-free.

Algarve is the most important citrus region of Portugal, with an area of 18,000 ha. The region is considered free of tristeza, as is the rest of Portugal. Therefore, in 1994 a systematic survey based on ELISA detection was initiated. One sample was collected for each 1.5 hectare of citrus area. We analyzed 3,500 samples and some foci of CTV were detected, all of them on topworked orchards. It was verified that this material was introduced from other countries, principally from Spain by illegal budwood introductions. It was also verified that orchards surrounding the CTV focus were not infected. However, older orchards are usually infected with other pathogens, like the psorosis complex, concave gum and impietratura and exocortis. Nevertheless, today this situation is changing with the supply of quality budwood to the nurseries.

Our work group is integrated by:

- * Beatriz Bonacalza (CdC - Virology)
- * Celso Mendes (DRAALG - Virology Laboratory responsible)
- * Josefina Mariano (DRAALG - Agriculture Head Director
/CdC Administration Council President - 1991/99)
- * Joaquim Neto Martins (CdC Administration - 2000)
- * Maria José Pinto (CdC -Pest Management)
- * Olimpio Sequeira Marques (CdC - Greenhouse and Production * * Rivelino Moreno (CdC - Greenhouse staff)



RAY YOKOMI - CALIFORNIA

The plan for the 14th Proceedings of the IOCV is to get everything to the printer (Painter) for galley in the next two months and the Proceedings out this fall.

Research - Research is continuing with characterization of CTV isolates that are spreading in Central California. This includes virulence based on host reaction in citrus indicator plants in the greenhouse, molecular evaluation, and aphid transmission. Research also continues on biological control of citrus aphids. Serdar Satar, a Ph.D. student from University of Cukurova, Balcali, Adana, Turkey, will arrive June 30, 2000 to begin a 6-12 month study of citrus aphid parasitoids in my laboratory.

MARCOS MACHADO - BRAZIL

Finishing the Genome Project of Xylella fastidiosa in Brazil. This is the first complete genome project of a plant pathogen in the world: the genome project of the causal agent of citrus variegated chlorosis (CVC), the bacterium *Xylella fastidiosa*. It represented efforts of 34 laboratories in the State of Sao Paulo (Brazil), funded by FAPESP (Fundacao de Amparo a Pesquisa do Estado de Sao Paulo) and Fundecitrus (Fundo Paulista de Defesa da Citricultura). After two years of activities, the genome project is finished. With 2,679,305 bp the circular chromosome, together with two plasmids, allowed us to evaluate 2,904 ORFs. Several genes correlated to pathogenicity were detected, including proteases, cellulases, synthesis of EPS, etc. The annotation has revealed

important characteristics of the bacterium, many of them probably correlated to pathogenicity. Functional genomic projects, using the information of the genome, have pointed out its pathogenicity looking for a better understanding on the relationship between the sweet orange, the bacterium, and its vectors. The Citriculture Center of Agronomic Institute of Campinas is participating in both the Genome and functional projects related to *X. fastidiosa*. For more information contact the website at <<http://www.fapesp.br>>. The paper on this project was accepted in Nature.

NORMA COSTA - ARGENTINA

In February 2000, Dr. Nuria Duran-Vila from IVIA, Spain, spent 15 days at INTA Concordia with Maria Ines Plata and Norma Costa setting up the equipment and teaching the know how for viroid detection with sPAGE, Slot-Blot and Imprint hybridization. This was a part of the PROCITRUS, Sanitary Citrus Program for Argentina. Three graduate students from the Univ. Nac. de Quilmes (Prov. of Bs. As.) and Alicia Nieto, from the Instituto de Fitovirologia y Virologia (INTA) in Cordoba, Argentina, also participated.



Nuria teaching us how to prepare a special buffer, "Agua de Valencia". Nuria in the laboratory of INTA Concordia. Present are Alicia Nieto, Nicolas Marchetta, Gabriel Iglesias, Diego Faccone, Norma Costa, Nuria Duran-Vila, Maria Ines Plata, and Fabian Ramos (greenhouse technician).

GERD MULLER AND SERGIO A. DE CARVALHO - BRAZIL

Woody Gall symptoms in different citrus species and varieties artificially colonized by the citrus brown aphid Toxoptera citricida.



Seedlings of 41 different citrus species and varieties normally used as rootstocks, were massively colonized with the brown citrus aphid *Toxoptera citricida* Kirk, obtained from Pera sweet orange trees showing symptoms of the Capao Bonito complex of CTV in Capao Bonito County, Southwest Sao Paulo State. Afterwards the plants were transplanted to a field nursery. The objective was to evaluate varietal resistance or tolerance to that virus complex. A survey carried out eight months after inoculation showed no stem pitting in the plants. However, the presence of galls similar to those induced by the vein enation/woody gall disease were observed in 73% of the plants of Volkamer cv 'Palermo'; (Photo left) 60% of Volkamer cv 'Catania2'; 20% of the Rangpur Lime; 13% of Volkamer cv 'Australian Red', a Rangpur lime hybrid of Orlando tangelo and Florida Rough lemon, and 7% of Carrizo citrange. The highest incidence and the largest gall size were observed in Volkamer cv 'Palermo' showing that this clone would be the most suitable to be used as an indicator plant in biological indexing tests for the disease. There are no previous reports in the literature about the occurrence of woody galls in Orlando tangelo and Carrizo citrange. These results confirm previous observations, that the pathogen is widespread in the citrus areas of Sao Paulo, and strengthen the necessity to include indexing for woody gall in the Sao Paulo citrus certification scheme.

TIM GOTTWALD HONORED.

Seven members of the American Phytopathological Society were honored as fellows of the Society at the 1999 APS meeting in Montreal, Quebec, Canada. Our own Tim Gottwald was one of the seven and there is a nice picture and write up of him in the January, 2000 issue of Phytopathology.

PAULO SERGIO TORRES BRIOSO - BRASIL

I began my scientific life in 1979, after graduating in agronomic engineering and was hired as Professor of Plant Pathology in the Department of Entomology and Plant Pathology, Institute of Biology, at Universidade Federal Rural do Rio de Janeiro (UFRRJ). My research and major published articles are on etiology, taxonomy, epidemiology and control of plant pathogens (especially viruses, viroids and phytoplasmas) that affect crops of economic importance in the State of Rio de Janeiro, such as vegetables, leguminous and fruit crops. I obtained my Master's degree in Plant Pathology at the Universidade de Brasilia, under the supervision of Dr. Elliot Watanabe Kitajima, and my Doctorate on Genetics, at the Universidade Federal do Rio de Janeiro. In 1994, a project on citrus leprosis in the State of Rio de Janeiro was initiated with the collaboration of Drs. Joao Pedro Pimentel, Luciana Pozzer, Helena Guglielmi Montano and biologist MSc. Marcos de Bonis, agronomic engineer Jadier de Oliveira Cunha Jr., We are coordinated with Dr. Gerd W. Muller and Dr. Marcos Antonio Machado. At present, citrus leprosis is the major virus disease of citrus in the State of Rio de Janeiro. Until now, no investigations on the etiology, taxonomy, epidemiology and control of the pathogen in the State of Rio de Janeiro had been carried out, and the information available had been based on symptomatology. Our research was initiated by surveying the vector *Brevipalpus phoenicis*, the only species present in the field. We observed that the vector population behavior is similar to that reported in the State of Sao Paulo. Under laboratory conditions we achieved rearing of colonies of *B. phoenicis* on potato tubers and on green bean pods, facilitating biological control studies. In ultrathin section from Seleta orange we demonstrated the presence of putative virus particles restricted to the cellular cytoplasm, and absent from the nucleus. This characterizes the virus (present in Rio de Janeiro) as a probable cytorhabdovirus, and not a nucleorhabdovirus, as previously reported by E. W. Kitajima and A. Colariccio. Another interesting feature is the mechanical inoculation of plant sap from fruit lesions, leaves and branches, which in dilutions higher than 100 fold do not induce symptoms (small necrotic local lesions - pencil tip-like lesions) on *Chenopodium amaranticolor*. On the other hand, dilutions below this value induce lesions on *C. amaranticolor*. Such lesions are much smaller than those reported by A. Colariccio. Currently, we are focusing our research on extracting the virus genome and producing specific primer pairs to be used in RT-PCR. Preliminary data indicates that vector control, under laboratory conditions, is feasible by using fungal isolates that infect bovine mites (*Boophilus microplus*). Thus far, the available data appears to support the likelihood that distinct viruses may be disseminated by the same vector, or that there exist a mixture of populations of *B. phoenicis* with different transmission specificity, for each of the virus species involved in citrus leprosis.



Leprosis symptoms on fruit and leaves.

Leprosis is one of the most serious diseases now occurring in Brazil and other South American countries. The high cost of spraying forces many farmers to abandon their citrus orchards. (Photo by R. F. Lee)

ROCHA-PENA - MEXICO

The brown citrus aphid (BrCA) *Toxoptera citricida* has recently moved into Mexican territory in February, 2000. According to the Secretaria de Agricultura, Ganaderia and Desarrollo Rural, there are two areas of infestation: one is in Chetumal, in the State of Quintana Roo and just across the border from Belize. The second area of infestation is northeast of the Peninsula including part of the State of Quintana Roo and the State of Yucatan. Thus far the BrCA infestations have not been detected in the main commercial citrus areas of Yucatan and Campeche. The most evident populations have been found on sour orange trees, that are common in back yards and in the countryside in many parts of the Peninsula.

JAMAICA

The photograph below was found in the office of the United Estates Limited, at Bog Walk, Jamaica and shows what happens when non-certified budwood was used in field plantings. What we see are the ravages of exocortis on sweet orange grafted on Carrizo citrange rootstock..



Peter McConnell and his fine staff led by Mrs. Grace Allen (left) have developed an outstanding indexing and certification program for Jamaica and have designed greenhouse facilities under warm and tropical conditions which will detect cool temperature graft-transmissible pathogens such as psorosis, concave gum, tristeza, tatterleaf etc.

With the brown citrus aphid now present in Central America and Mexico there will be a rush to plant citrus on tristeza-tolerant rootstocks such as Carrizo or Troyer citrange. Without a certification and indexing program the results will surely look like the photograph below. It would be advisable to visit the facilities at Bog Walk, Jamaica to study the excellent program developed there.

**Membership Application in the
INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS**

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 14th and 15th conference of the IOCV (For the three year period 1998-2001)

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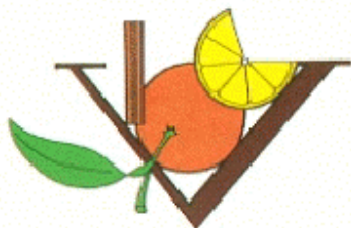
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Dept. Plant Pathology
University of California
Riverside, CA 92521-0122 USA



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