

INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

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IOCV NEWSLETTER

January 2004

FROM THE CHAIRMAN

Pedro Moreno

Dear Friends and colleagues,

The end of 2003 gives me the feeling that this year has accumulated many traits of "annus horribilis", considering the number of natural and man-made disasters that have occurred in the world during this period and the significant losses suffered by our organization. Now that a New Year is starting let me renew my best wishes for peace, happiness and a successful work for all of you during 2004.

One item of good news of the past period is that the Proceedings of the 15th Conference has finally arrived and the volume looks really beautiful. The Wallace Award Committee has started evaluation of full papers to discuss if one or more of those deserve the award.

In this regard, I have to announce some news for the next IOCV meeting. The Dave Gumpf family has expressed his will of dedicating the Dave Gumpf memorial fund to support and encourage IOCV research on diagnostic of citrus diseases and certification (the two main topics to which Dave dedicated his efforts). This is to be done by creating a new award for the best paper on these topics to be presented in future IOCV conferences. The Board of Directors have agreed that this can be a good idea to honor the memory of our past treasurer and friend and to motivate research and presentation of papers on these important topics in future conferences. The Board will present a motion at the next IOCV business meeting (in Mexico) to approve the creation of the Dave Gumpf Award, and if approved, it would be applied to papers presented in this upcoming conference. The Wallace Award Committee nominated for the upcoming conference would evaluate papers for both prizes, which could eventually be assigned to the same paper, to different papers or perhaps left empty if no outstanding paper is presented in that conference.

Mario Rocha and John da Graça continue their active preparation of the 16th IOCV Conference in Monterrey, México, with pre-conference and post-conference field visits in Weslaco (Texas) and Veracruz (México), respectively. An update with additional details is provided in this Newsletter. I encourage all those who have not yet answered the first circular for pre-registration to do it as soon as possible so that the organizers can do better planning for lodging and transportation.

Hopefully, In the last two months you had received a message from Chester Roistacher announcing an inventory reduction sale of the IOCV Proceedings of the 7th to 15th Conferences. The reason for this offer is that a number of

volumes of these issues were still stored in Riverside, and it was felt that they should better be stored in your own library or your Department library. The Board of Directors decided that it was time to offer an opportunity to IOCV members who could not attend one or more of those conferences, to complete their collection of Proceedings at a substantially reduced price. This also could contribute to keep the storage surface at a more reasonable level and put our books closer to their objective: to be read and used! (See enclosed application form)

Finally, since we are now about 9 months from the next IOCV conference I would like to remind you about two important questions:

1) The Conference Location Committee is searching for potential sites to celebrate the XVII IOCV Conference in 2007. Several possibilities are being considered, but no formal offer has been received so far. I greatly encourage any IOCV member who would like to host this conference in his country to contact members of this committee (Pat Barkley: pat.barkley@bigpond.com; Josy Bové: <josy.bove@libertysurf.fr>; Steve Garnsey: <sgarnsey@worldnet.att.net>; Luis Navarro: <lnavarro@ivia.es>; John da Graça: <jdgraca@ag.tamu.edu>, or myself: <pmoreno@ivia.es>) as soon as possible, so that the offer can be studied and eventually presented for approval at the business meeting

2) The new IOCV By-Laws approved during the past conference in Cyprus, considered in its Section 3 the possibility of granting "the honour of Fellow of the IOCV for lifetime achievement to persons who have contributed significantly to the growth, welfare, functions or activities of IOCV including service to the organization, sustained scientific contributions and otherwise furthering the objectives of IOCV". In the same section it is stated that IOCV members may submit to the Chairperson (Pedro Moreno <pmoreno@ivia.es>) any supporting documents or information on persons to be considered for this honour. The Board of Directors shall constitute a committee to consider the granting of Fellowships, whose final nomination has to be approved at a business session. IOCV has presently no Fellow, even if it has some members who are competent scientists and have contributed for many years to IOCV objectives. I think that it is the time to consider some nomination and I encourage you to send documented proposals on potential candidates, if possible, supported by more than one member.

CITRUS LEPROSIS VIRUS IN RIO DE JANEIRO

Professor Paulo Sergio Torres Brioso – UFRRJ, Brasil

In 1994, a project on citrus leprosis in the state of Rio de Janeiro was initiated with the collaboration of Dr. João Pedro Pimentel, Dr. Luciana Pozzer, Dr. Helena Guglielmi Montano, Dr. Marcia Cristina Campos de Oliveira, MSc. Daniel Vazquez Figueiredo, MSc. Jadier de Oliveira Cunha Junior, MSc. Maria do Socorro da Rocha Nogueira.

At present, citrus leprosis is the major virus disease on citrus crop, in the state of Rio de Janeiro.

Until 1994, 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 was based on symptomatology.

Research was initiated by surveying the vector *Brevipalpus phoenicis* (the only present species) in the field. We observed that the vector population behavior is similar to that reported in the state of São Paulo.

Under our laboratory conditions we were able to rear colonies of *B. phoenicis* on potato tubers and on green bean pods, facilitating biological control studies.

Outstanding differences have been observed for the pathogen, in ultrathin cuts from Seleta orange, demonstrating the presence of putative virus particles restricted to the cellular cytoplasm, and absent from the nucleus. This characterizes the virus (present in Rio do Janeiro) as a probable cytorhabdovirus, and not a nucleorhabdovirus, as previously reported by E. W. Kitajima and A. Colariccio.

Another interesting feature regards mechanical inoculation of plant sap from fruit, leaves and branches lesions, which, in dilutions below 100 fold, induces symptoms (small necrotic local lesions - pencil tip-like lesions) on *Chenopodium amaranticolor*. These differ in number dependent on the citrus cultivars (The highest number of lesions developed in the employed dilutions was obtained from 'Seleta' – Fig. 1, an intermediate value from 'Pêra' and the lowest number from 'Lima'. Such lesions are much smaller than those reported by A. Colariccio.

Jadier de Oliveira Cunha Jr. in his thesis (under my orientation) using degenerated primers had amplified fragments of the CiLV (cytorhabdovirus) genoma from RNA extracted from leaves of citrus and *Chenopodium* sp.

Currently, we focus our research on cloning the amplified fragment of the CiLV (cytorhabdovirus) 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*).

So far, 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.



Fig. 1. Isolate of 'Seleta' inoculated in leaf of *C. Amaranticolor* (several lesions) – 10^{-1} dilution .

From Italy

Oswaldo Lovisolò

I retired and I do not have my old e-mail address at the "Torino Istituto di Virologia Vegetale". My new address is o.lovisolo@virgilio.it.

The last experimental work was the one done with Italian and Brazilian colleagues on citrus tatter leaf virus. The results are published in the paper: LOVISOLO, O., ACCOTTO, G.P., MASENGA, V. & COLARICCIO, A. An isolate of Apple stem grooving virus associated with Cleopatra mandarin fruit intumescence. *Fitopatologia Brasileira*, 28(1): 54-58.

The new international virus nomenclature based on genetic data has certainly the advantage of putting in evidence of virus relationships previously unsuspected. However it is creating new problems on quarantine legislation. The origin of some virus diseases in woody plants could have happened in special conditions not easily repeatable. It is people who spread the virus with agamic propagation, especially when no vectors are present. In the case of CTLV isolates of the Apple stem grooving virus (ASGV), quarantine authorities may omit to take proper precautions against the introduction or further spread of CTLV isolates in citrus, when in the country ASGV isolates are present in Rosaceae.

FROM CALIFORNIA

Marylou Polek

Central California Tristeza Eradication Agency

The Central California Tristeza Eradication Agency (CCTEA) continues with its mission to suppress CTV in commercial citrus groves in designated areas of the Central Valley. According to the current operational plan, all commercial citrus within the participating pest control districts is surveyed on a 5-year subsampling cycle with 20% of the total acreage targeted each year. The Hierarchical Subsampling Method, devised by Gottwald and Hughes, remains as the approved survey method. In September 2003, the overall infection level of the mandated tree removal area was estimated to be 0.12%.

When a grove has suspect positive trees, it is placed on a Collection Priority List (CPL) which is sorted from highest to lowest estimated percent infection and updated after each collection season (fall and spring). Each year within financial constraints, approximately 2,500 acres are selected from the top of this list and sampled on a single tree basis to identify and subsequently remove infected trees. This plan has managed to address the estimated level of infection in suspect positive groves down to 0.86%. During 2003, 1,761 CTV-positive trees were removed. As part of the tree removal process, a backhoe is used to remove the root system to prevent re-growth. Where access with large equipment is not possible, staff is experimenting with injections of herbicides into the stump.

Most likely due to atypical weather conditions, the virus titer and tree flush were below normal this past fall and made survey work challenging. The 2003-2004 fiscal year's operational goals are behind schedule and it is hoped that conditions in the spring will permit completion of all targeted acreage. With the increasing amount of acreage planted to mandarin varieties, Agency laboratory staff is researching the phenomena of high background on field grown mandarin varieties in the ELISA process. Healthy greenhouse controls do not demonstrate this condition even when they were propagated from field trees that did. Field trees grown at the Agency and the University of California Lindcove Research and Extension Center are being monitored weekly to examine physiological reactions in response to ambient weather conditions.

State Testing Program

The statewide program managed by the California Department of Food and Agriculture (CDFA) that registers and tests all trees used for commercial propagation is now conducted at the Central California Tristeza Eradication Agency under the direction of Dr. MaryLou Polek. This program ensures that all source trees used to produce new citrus trees are free of Citrus tristeza virus. In addition, trees are also tested for Citrus psorosis virus, Citrus exocortis viroid, and other viroids. Dr. Robert Krueger, USDA, ARS is making good progress on improving diagnostic methods for citrus stubborn. Once his methods are approved by the CDFA, registered trees will be tested for this pathogen also.

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126	CACHEXIA - FRENCH
88	CANKER IN THE MALDIVES
38	CITRUS CHLOROTIC DWARF
57	CONCAVE GUM
124	CONCAVE GUM - FRENCH
20	CRISTACORTIS
125	EGYPT CERTIFICATION PROGRAM
75	EXOCORTIS
106	GREENHOUSE AS A PLANT LABORATORY
85	GREENING - BROCHURE
58	GUM POCKET
131	GUM POCKET - FRENCH
44	GUMMY BARK
55	IMPIETRATURA
73	INOCULATION TECHNIQUES
68	INFECTIOUS VARIEGATION
127	INFECTIOUS VARIEGATION - FRENCH
117	KRUEGER - GERMLASM REPOSITORY
64	LEPROSIS
118	MANDATORY CERTIFICATION
79	NAVEL - PARENT - HISTORY
65	PSOROSIS PART 1 - SYMPTOMS
129	PSOROSIS PART 1 - SYMPTOMS - FRENCH
66	PSOROSIS PART 2 - INDEXING
130	PSOROSIS PART 2 - INDEXING - FRENCH
9	RICHARD LEE - CVC
47	RICHARD LEE - MANDATORY CERTIF.
14	RICHARD LEE - TRISTEZA
36	RICHARD LEE - WITCHES BROOM
61	SATSUMA DWARF
87	STUBBORN
143	SHOOT TIP GRAFTING IN VITRO
141	SUDDEN DEATH DISEASE BRAZIL
72	TATTERLEAF
42	THERMOTHERAPY
97	TRISTEZA - PART 1 - HISTORY
98	TRISTEZA - PART 2 - THE VIRUS
102	TRISTEZA - PART 3 - EPIDEMIOLOGY
103	TRISTEZA - PART 4 - CROSS PROTECTION
142	TRISTEZA - PART 5 - PERU X-PROTECTION
82	UC SYSTEM FOR PLANT GROWTH
60	VEIN ENATION
56	WINTER SUPPLEMENTAL LIGHTING
77	WOOD POCKET - UPDATE MEXICO

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News from the IVIA, Moncada, Valencia, Spain

Mari Carmen Vives, José Antonio Pina, Pedro Moreno, Luis Navarro and Pepe Guerri

In recent years we characterized biologically (1) and molecularly (2, 3, 4, 5) Citrus leaf blotch virus (CLBV), a citrus virus detected in samples from at least Corsica (France), Valencia (Spain), Florida and California (USA), Japan and Australia. Biological and molecular differences with other known viruses support the idea of including it in a new genus for which we proposed the name Citrivirus (3). CLBV has been found associated with the presence of bud union crease on trifoliolate rootstocks, mainly citranges and citrumelos. However, presently it is difficult to assess the economic importance of CLBV, since so far we have been unable to prove that it is actually the causal agent of bud union crease (1, 6, 7). The virus can be detected by inoculation to Dweet tangor seedlings incubated at 18-25°C. Sequencing of the CLBV genomic RNA allowed development of quick detection procedures based on molecular hybridization and RT-PCR (6, 7). The primers, probes and protocols used for detection are described in these two papers, but additional details can be provided to anyone interested. Both procedures require taking at least six samples from the candidate tree to reduce the risk of false negatives due to low virus titer and uneven distribution within infected plants. Even with this limitation, laboratory procedures are preferred, since they are faster, cheaper and more reliable than biological indexing. A minimum of six indicator plants graft-inoculated with at least four bark pieces each are recommended to overcome diagnosis problems derived from uneven virus distribution. Titer seems to be higher in regions close to the apical meristems, which might explain difficulties to eliminate this pathogen by shoot-tip grafting in vitro (1). Recently we have observed that symptoms induced by CLBV are indistinguishable from those caused in the same conditions by two sources of Dweet mottle, a disease described by Roistacher and Blue (8), reported only in California. CLBV was indeed detected in both sources by RT-PCR. As CLBV, dweet mottle was also difficult to eliminate by thermotherapy and by shoot-tip grafting in vitro. These findings suggest that dweet mottle syndrome might be caused by CLBV and experiments to fulfil Koch's postulates are in course.

The new diagnosis tools have also facilitated studies on potential seed transmission, since they allow rapid detection of CLBV in many symptomless young plants. For this purpose we grew 120 to 210 seeds from CLBV-infected Troyer citrange, Nagami

kumquat and sour orange in the greenhouse and individual 4-month-old seedlings were analyzed for CLBV by RT-PCR. About 2.5% of the seedlings of each species reacted positively and infection was later confirmed by biological indexing on Dweet tangor (9). This finding will have serious implications in quarantine and certification programmes for pathogen free plants. Up to now citrus seeds were considered a safe material in plant propagation and germplasm movement, despite limited evidence that suggested occasional seed transmission of some agent inducing young leaf patterns. The new data clearly indicate that control of CLBV spread during citrus propagation requires, not only virus-free buds, but also rootstock seedlings that originate from CLBV-free seed source trees. Therefore, regulations of citrus certification programs may need to be changed to include periodical control of seed source trees to be sure that they remain CLBV-free. Incorporation of these measures to the Spanish certification programme have started and likely they will be also introduced in programmes of other places like Australia and Florida. Also international regulations for citrus seed movement likely will have to be modified to include a phytosanitary certification indicating that seeds have been collected from CLBV-free trees.

References. (1) Galipienso et al. 2000. *Plant Pathol.* 49: 308-314. (2) Galipienso et al. 2001. *Arch. Virol.* 146: 357-368. (3) Vives et al. 2001. *Virology* 287: 226-233. (4) Vives et al. 2002. *Virology* 295: 328-336. (5) Vives et al. 2002. *J. Gen. Virol.* 83: 2587-2591. (6) Vives et al. 2002. *Proc. 15th Conf. IOCV*: 205-212. (7) Galipienso et.al 2004. *Eur. J. Plant Pathol.* 110: (in press). (8) Roistacher and Blue 1968. *Proc. 4th Conf. IOCV*: 13-18. (9) Guerri et al. 2004. *Plant Dis.* (in press).

RICHARD LEE NOW IN CALIFORNIA

USDA-ARS National Clonal Germplasm Repository for Citrus & Dates, Riverside, Calif

Robert Krueger

The Repository welcomes Dr Richard Lee as Research Plant Physiologist/Research Leader. Dr Lee is well known to many IOCV members due to his many years of service to the organization as well as his career at the University of Florida, FAO, and as a consultant world-wide. The Repository staff will benefit greatly from Dr Lee's years of experience.

Dr Benjamin Rangel of the Repository is continuing his work on the refinement of the stubborn assay and analysis of genetic diversity of *Spiroplasma citri* in California. The Repository continues its investigations in other horticultural areas and to serve as a source of pathogen-tested budwood. Recently, the Repository has served as a cooperative resource to Mexican phytosanitary authorities in their efforts to establish an importation/sanitation program.

