INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

IOCV NEWSLETTER **January, 2000**

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FROM THE CHAIRMAN

Dear Friends and Colleagues,

Happy New Year. I hope this year will be a happy and successful one for you.

It is gratifying to see that so many of you have contributed to this Newsletter and are willing to convey what you are doing and what you have published recently. Some of you are using the Newsletter as a medium to seek information, and I urge those of you who can help, to do so. I thank Chet. for all the hard work he puts in to the Newsletter.

Anastasia Kyriakou informs me that preparations are well underway for the next IOCV Conference in Cyprus in 2001. Moshe Bar Joseph has held discussions with local travel agents about a trip of 8-9 days, to include Israel and Jordan, at an estimated cost of 110-120 US $ /day. He suggests this as a pre-conference tour, with visits to some interesting citrus sites in Israel, combined with some nice tourist attractions in Israel and Jordan.

Ray Yokomi, John da Graca and Richard Lee are continuing with the unenviable job of editing the proceedings of the last conference. John says they have received around 75 manuscripts (full length and short communications), of which more than 40 have been revised. Where papers have not been submitted, edited abstracts will be published.

If any of you have suggestions for improving IOCV, please let us know.

With best wishes,

Pat (Broadbent) Barkley.

ECOPORT.COM

(Richard Lee)

EcoPort (, a service to global ecology and biodiversity management, went public on January 1, 2000. It exists under the custodial auspices of the University of Florida, the National Museum of Natural History at the Smithsonian Institute, and the Food and Agricultural Organization (FAO) of the United Nations. EcoPort is dedicated to "consilience", the process of building integrated, relational linkages between various domains of information necessary to manage biodiversity. EcoPort is, essentially, a communally-owned database on the internet. Users share their data and expertise which they place in a publically accessible, open-source pool of information for common use. Every person who contributes personal knowledge, gains access to the sum of all similar contributions. Individuals can write narratives that use other people's inputs. All contributions and resources, remain the property of the original contributor and are displayed under that person's name, logo or copyright statement. Thus, in EcoPort, finding information, creating knowledge, teaching and learning and solving problems, becomes a single, seamless process: digital teamwork fueling and driving a consilience engine.

EcoPort's sharing philosophy and the set of tools and procedures to deliver its consilient services to the public, has proved itself by focusing on a limited subject area. During 1999, an EcoPort precursor system, called GPPIS (the Global Plant and Protection Information System) was operated by one of the partners of the present consortium, FAO. EcoPort has the flexibility to mange ecological information. An insect can be described as a pollinator, a pest, or a hyperparasite.

EcoPort includes a bibliographic reference resource which can be searched by author, title, and/or year; glossaries; hypermemes which can be used to present method protocols complete with illustrations and annotations; taxonomy keys; slide shows; and a picture databank.

IOCV members are invited to adopt a pest or plant of their choice, and to contribute their information. If all your references are not included in the bibliographic reference resource, contribute the ones which are missing. Watch for the IOCV home page under the affiliates section of EcoPort.

ECOPORT SLIDE SHOWS

(Chet Roistacher)

IOCV membership should be aware that there is a Website 2000 readily accessible which contains an unusual amount of information pertinent to citrus virus and virus-like diseases. This is `EcoPort'. It is a data base and not just a Website. It is designed and devoted entirely to inter-disciplinary integration of information to manage biodiversity. Ecoport is sponsored by the Food and Agricultural Organization of the United Nations (FAO), The University of Florida and the Smithsonian National Museum of Natural History. EcoPort is a most exciting and informative concept, easy to access and explore and contains a world of information. As of January, 2000 there are almost half a million bibliographical references, over 27,000 Glossary of Terms, over 6,000 pictures with over 1,000 waiting to go in and 43 slide shows. Slide shows of which should be of interest to IOCV members are as follows:

CURRENT SLIDE SHOWS PERTAINING TO CITRUS FOUND ON ECOPORT/RESOURCES/SLIDE SHOWS

Lecture Number	Slide shows
9	Citrus variegated chlorosis
14	Citrus tristeza virus
20	Cristacortis
36	Witches' broom
38	Citrus chlorotic dwarf
42	Thermotherapy
43	Evolution of pathogens
44	Gummy bark
47	Why mandatory certification of citrus?
52	Med fly
55	Impietratura
57	Concave gum
58	Gum pocket of trifoliate
60	Vein enation
61	Satsuma dwarf
64	Leprosis
65	Psorosis I - History & field symptoms
66	Psorosis II - Diagnostics and control
68	Infectious variegation
70	Abnormal bud union of sweet/rough lemon
72	Tatter leaf
73	Inoculation techniques
75	Exocortis
76	Cachexia

We are indebted to Tonie Putter and to Marco Marsella for this superb work and encourage IOCV membership to explore this site and report your observations and any thoughts for corrections and new ideas to Tonie Putter <Tony.Putter@fao.org> or to Marco Marsella <support@ecoport.org>.

Tonie Putter recently wrote an introduction to Ecoport as follows: "Light a candle - don't just curse the darkness... The EcoPort service focuses on the process of sharing information. A year ago, we had 2000 pictures and 10,000 species names listed in the database. In January 2000, we have over 6,000 pictures and about 40,000 species. These species records are largely empty inasmuch as they contain only names as in standard checklists, but a named record for a species is the minimum requirement for data accumulation and integration.

Every time a user like you finds a mistake, or does not find information in EcoPort, there are two choices: complain and walk away or share the information when you do find it. The people who chose the latter option were responsible for increasing the data in EcoPort during 1999. A person who shared just one picture a year ago, now has access to 6,000 pictures taken by other biologists, a ratio of effort: reward of 1:6,000. You can have something

for nothing because EcoPort is a public service dedicated to ecology education, but imagine how much better you will serve your self-interest by sharing.

On average, over the last 6 months of 1999, 100 technical, data-level improvements per day were made to the shared EcoPort database by the global network of biologists who used their passwords to write ecology knowledge into the communal EcoPort database. You are invited to join the EcoPort community".

On your server type <ecoport.org> and open a new world of information.

NEWS FROM THE MEMBERSHIP

PAT BARKLEY - AUSTRALIA

ZHOU CHANGYONG from the Citrus Research Institute, Beibei, People's Republic of China, is now in the final year of his Ph.D. thesis on tristeza mild strain cross protection through the University of Sydney. Experiments are being carried out at Elizabeth Macarthur Agricultural Institute (EMAI), NSW Agriculture with DEB HAILSTONES AND PAT BARKLEY. Zhou is funded by a John Allwright Fellowship from Australian Centre for International Agricultural Research (ACIAR). Evidence based on the sequencing of selected genes (from bud isolates and single aphid transmissions) and biological indexing strongly suggests that the mild CTV strain that has protected Marsh grapefruit against CTV stem pitting in Australia for the last 30 years is a pure strain (population). 6 monoclonal antibodies have been screened for their abilities to differentiate this mild pre-immunizing strain from severe strains of tristeza, but no clear differentiation has been obtained. The distribution and movement of different strains of tristeza have been monitored within plants of a few citrus species by DTBIA, RT-PCR and RFLP. Pre-immunized plants challenged by a severe CTV isolate have been monitored by multiplex RT-PCR and/or RFLP with interesting results.

RACHAEL CONNOR is in the last year of her part-time Masters degree, under the supervision of *Deb Hailstones, Pat Barkley and John Bowyer* (University of Sydney). Rachael's work is progressing very well and focuses on molecular methods to distinguish strains of CTV and to analyse the genetic variation within the Australian CTV population. Approaches used include the RT-PCR of selected genomic targets, followed by RFLP, SSCP, sequencing and the design of discriminatory oligonucleotides for use in molecular screening.

DEB HAILSTONES and PAT BARKLEY report the development of a new molecular test for citrus tatter leaf virus. It uses a semi-nested RT-PCR approach and provides more than 500 times the sensitivity of ELISA-based techniques. An abstract of the procedure was presented at the conference of the Australasian Plant Pathology Society in Canberra in September 1999, and a full manuscript is about to be submitted to Australasian Plant Pathology.

RON HUTTON, PAT (BROADBENT) BARKLEY AND KEN BEVINGTON have published a review article on viroid dwarfing in the journal, Horticultural Reviews which summarises thirty years of Australian research on viroid dwarfing in citrus in high density plantings.

Hutton, R.J., P. Broadbent and K.B. Bevington (2000). Viroid Dwarfing for High Density Plantings. Horticultural Reviews 24: 277-316.

Three scientists (TANG KEZHI, HUANG SEN and YANG FANGYUN) from the Citrus Virus Disease Research Group of Citrus Research Institute of CAAS at Beibei, Chongqing, P.R. China, visited EMAI for further training by DEB HAILSTONES in molecular detection methods for citrus pathogens and in glasshouse management. The training was partly sponsored by ACIAR.

The Australian Citrus Budwood and Seed Scheme, managed by AUSCITRUS (an industry body with technical support from NSW Agriculture) is undergoing major changes to meet industry changes brought about by the move to increased export of fresh fruit and the demand for new varieties. PAUL FLORISSEN has been appointed to manage the field operations of the Scheme at Dareton and interviews have recently been carried out for an Industry Development Officer. The move to new varieties e.g. Navelina, required a shift from budwood supply from field grown trees to rapid nursery multiplication. This was initially carried out by nurserymen, but proved unpopular, as nurserymen in general failed to meet demand and Auscitrus was losing control of the budwood scheme. (There is no government legislation to ensure usage of the scheme). Auscitrus is now carrying out rapid nursery multiplication of those varieties for which budwood is in short supply and is working with the nursery industry to develop an industry operated nursery accreditation scheme. High demand varieties are also established in field multiplication plantings as soon as possible after release from quarantine. Post entry quarantine (with shoot tip grafting and disease indexing) of newly imported varieties is carried out by the Australian Quarantine and Inspection Service at the Plant Quarantine Station at Eastern Creek (NSW). Virus-free citrus is maintained in an insect proof screenhouse at EMAI. Trees pre-immunized with the mild isolate of CTV

are maintained in insect-proof screenhouses at EMAI and Dareton for budwood supply for rapid nursery multiplication. Indexing (biological, serological and molecular) of field mother trees is carried out by CAROL DEPHOFF and others at EMAI. The scheme is , in general, working well, but orange stem pitting in Queensland continues to be an impotant industry problem. PAT BARKLEY and NERIDA DONOVAN have begun mild strain cross protection trials.

Y. S. AHLAWAT - INDIA

Our research on citrus virology has been further strengthened with a megaproject by the world bank under the National Agricultural Research Project (NATP). The major objectives of the NATP are the production of diagnostics for citrus viruses prevalent in India like citrus ringspot and yellow mosaic. These diagnostic reagents will help the indexing programme in the country and will also be useful for plant quarantine organizations in India and elsewhere.

Another important activity of the research is to identify mild strains of CTV. Although CTV is very mild in Northern India because of absence of *Toxoptera citricida* and negligible field spread, it reacts strongly with MCA-13 suggesting it to be severe strain. I would like to have advise and comment on this.
Contact <ysa_pp@irai@ernet.in>.

Citrus yellow corky vein has been transmitted mechanically from Mexican lime to Mexican lime. Two viroids: exocortis and hop stunt have been found associated using roboprobes, but the exocortis reaction on Etrog citron was not observed. It appears that it may be a new strain of exocortis viroid. However, we are trying to establish if a virus is also involved and if the symptoms are caused by a synergistic effect of a viroid and a virus. We are getting some positive indications.

Our team: *N.K.Chakraborty, V.K.Baranwal, R.P.Pant, P. Ramachandran and Shelly Praveen* are developing good information on molecular aspects of citrus viruses and viroids. Three Ph.D. students are also working on CTV, CRSV and yellow mosaic. Hopefully they will come out with good useful information for citrus virologists.

Our recent publications are:

Rustem, F.A. and Y.S.Ahlawat. 1999.
Yellow corky vein: a viroid disease of citrus in India. Indian J. Agric. sci. 69(11):789-794.

Ghosh, D.K., A.K.Das, Shyam Singh, S.J.Singh and Y.S.Ahlawat. 1999.
Association of a phytoplasma with witches' broom - a new disease of acid lime (*Citrus aurantifolia*). Current Science. 77:174-177.

TERRY LYNN THOMPSON - BERMUDA

Bermuda has undergone some political changes, and the new Board of Agriculture is reviewing the existing Citrus Budwood Program. Presently, virus free citrus plants grown by the Department of Agriculture and Fisheries are released to growers and homeowners. In the worse case scenario (a common one I might add), these growers interplant the new, virus free plants with old virus infected citrus plants. In addition to this, this Department has released varieties grafted on new rootstocks such as Rangpur lime. With the old citrus comprising the majority of citrus trees in the landscape and the cultural methods employed here, it may only be a matter of time before viroids such as exocortis and cachexia reveal themselves. The new Board of Agriculture has expressed an interest in the possibility of the removal of virus-infected citrus and its replacement with clean material.

I am seeking advice on how to best approach this issue. Given the size of the citrus industry on the island and the dooryard nature of the plantings, do you think it is possible to eradicate the virus (or possible viruses) in the existing old citrus? Do you think that the removal of these old trees and their replacement with new, virus free citrus trees is a realistic option? If your response to either of the former questions is yes, my next consideration is how best to educate the public on the pros and cons of managing or eradicating citrus viruses in Bermuda. I think if the public can be convinced our actions are in their best interest, and if they can be persuaded to work with us, the process of tree removal can be conducted in a most amicable way. If we decide that the removal of these trees is a viable option, the Government is considering providing replacement trees for those that have been removed.
<plant@ibl.bm>

JOHN DA GRACA - TEXAS

I spent a week in Portugal and 1 day in Madrid as a technical advisor for the FAO which is supervising the research on CTV detection and strain identification being done mainly by *Gustavo Nolasco* in Faro. I spent some time with him, and also visited *Fernando Pons, Vicente Torres* and *Flora Sanchez* of INIA in Madrid. They are collaborators in the project. *Paul Pilkauskas* of FAO, *Siegfried Hirsch* from the Common Fund for Commodities in Amsterdam (they are funding this project) and his technical advisor *John Pegus* from the Ministry of Agriculture in Trinidad. While In Faro, *Gustavo Nolasco* gave me a copy of a paper on his work.

Nolasco et al. 1997. Sensitive CTV diagnosis using RT-PCR and an exonuclease fluorescent probe assay Fruits 52:391-396;

Nolasco et al. 1999. PCR based detection and strain typing of citrus tristeza virus. Petria 9:135-135.

We are now making progress on the IOCV proceedings. About 70 papers have been submitted (around

37 back in revised form). Quite a few have requested just the abstract, and several have not responded to my requests so we will just do their abstracts. Richard Lee is now working on his editing, so we should be ready to go in early 2000.

NURIA DURAN-VILA - SPAIN

Here are a few words on the activities in my lab. *Ana Palacio-Bielsa* who got a PhD in my laboratory will join *Dr. Semancik*'s laboratory on Jan 16. As an outcome of her work, two papers have been published and one is in the process of being reviewed.

Palacio, A., Duran-Vila, N. 1999.
Single strand conformation polymorphism (SSCP) analysis as a tool for viroid characterization. Journal of Virological Methods 77:27-36

Palacio, A., Foissac, X., Duran-Vila, N. 1999.
Indexing of citrus viroids by imprint hybridization. European Journal of Plant Pathology (to be issued in 1999).

***Monica Gandia, Mohammed Chaffai* (from Morocco) and *Zied Fadda* (from Palestine) are presently working on different aspects of my program on viroids. *Juan Romero Durban*, a former student has joined again our laboratory with a postdoctoral fellowship.**

We are keeping an excellent relationship with different institutes in South America. We have had several exchange visits: *Gabriela Pagliano* from the University of Montevideo spent one month at IVIA. *Marianela Soto* from CENSA in Havana (Cuba) attended a postgraduate course in Madrid and then joined my laboratory for 3 weeks. *Karelia Velazquez* from the Institute of Citrus and Other Fruit Trees in Havana (Cuba) spent 3 months in my laboratory with the aim of characterizing several viroid sources to be further assayed for dwarfing. *Chriantiane Barbosa* from EMBRAPA (Brazil) will join our group next February with the aim of getting a PhD.

Last September I had the opportunity to visit the Universidad Catolica de Valparaiso in Chile to give a short training on viroid indexing methods. I had the opportunity to see unusual cachexia or cachexia-like symptoms.

MICHAEL KESINGER - FLORIDA

Seventy-three commercial citrus nurseries were active in Florida this past year. The average commercial nursery propagated approximately 90,000 trees. Nursery propagations increased slightly in 1998-99 to 5.9 million trees. Forty-eight nurseries reported an increase in their amount of propagations. Twenty-five nurseries propagate 87% of the commercial citrus nursery trees in Florida. Five rootstocks account for 87% of all nursery propagations. The top five rootstocks listed in order are; Swingle Citrumelo, Carrizo Citrange, Volkamer Lemon, Cleopatra Mandarin and Smooth Flat Seville. The favorite scion varieties in Florida are Valencia, Hamlin and Midsweet in that order. Sweet oranges make up 89% of all propagations.

One of the biggest trends in Florida's nurseries is the shift toward increase blocks for propagating material. The number of increase block trees increased by 87% percent this year. Increase blocks now supply 32% of Florida's budwood. Screenhouses supply 6.6% of the State's budwood. The foundation screenhouse in Immokalee was doubled in size this summer with plans for future expansion in the works. As of yet no budwood has been distributed from this new facility. Once the Immokalee foundation screenhouse is operational the percentage of screen protected source material should be further increased. Several private nurseries have constructed or are planning future screenhouses.

The third annual citrus tristeza virus testing of field scion trees has been completed. With the exception of our Immokalee Foundation Grove, severe tristeza infection rates have averaged under 6%. Two commercial labs and four research laboratories were certified to run tristeza ELISA this year.

Citrus viroid testing in the bureau's laboratory and greenhouses has been on track to test all field budwood source trees every six years. Viroid testing of all trees is done with a combination citron greenhouse index and laboratory PCR tests.

The Bureau of Citrus Budwood Registration should have its own page up and running by the time you receive this newsletter. Look for the link to our page from our Division of Plant Industry web site at: http://doacs.state.fl.us/~pi/

CHARLES YOUTSEY - FLORIDA

Citrus nurserymen always seem to catch the brunt of the problems of diseases as they constantly pop up. The quarantine regulations or the threat of quarantine seems to be present in Florida as a constant risk to survival for those trying to grow citrus nursery stock. Citrus canker continues to pop up in unexpected places forcing nurserymen to install sanitation devices and security measures hoping to escape infection from movement of plants, people and equipment. Many are moving into greenhouse production to reduce the risk of early infection from aphid spread tristeza, wind born canker and the Diaprepes root weevil. All of these measures are expensive and put severe pressure on the bottom line, (PROFITS). However improvements in security and sanitation should result in a more efficient operation and combined with mandatory use of pathogen-free propagation material, produce a better quality product of increased value to the grower.

BOB MILNE, ITALY

The end of the old, bad, but comfortable millennium also saw the end of our 3-year European Community contract on tristeza and psorosis in South American and Mediterranean countries, headed by *Pedro Moreno*, with his skills of efficiency, rigor, and friendliness, combined with a capacity to bite sharply at the fleshy flanks of the Brussels bureaucracy when necessary (it often was).

Our part concerned the likely cause of psorosis, citrus psorosis virus, type species of the new genus Ophiovirus. Another known member and a further probable one of this new genus turn out to be soil-transmitted, very likely by *Olpidium brassicae*, so please place bets as to the REAL natural vector of psorosis!

Our main gain this last year was to produce and test two mouse monoclonals against the coat protein of psorosis virus, one of which (13C5) has so far detected all the isolates thrown at it, from the Mediterranean, South America and the USA. Time will tell exactly how universal this mab may be. The mabs were combined with our existing polyclonal rabbit antiserum in a robust and sensitive TAS ELISA that can easily detect the virus in field trees, even in poor, out of season or symptomless material (see Alioto et al 1999, Plant Pathology 48, 735-741). Preliminary trials at IVIA, Valencia, have confirmed an excellent correlation between biological indexing and ELISA results, and further trials are in progress there, in Argentina, and elsewhere, so the way is opening to use this system widely for detection and control.

***Daniela Alioto*, a prime mover in this work, now returned to Naples, continues energetically with ELISA tests for psorosis in southern Italy, and has found many young, still symptomless trees infected, especially sweet oranges and Clementines, though infection rates in older plantings are low. The infected material is mostly being introduced from Sicily. Daniela and *Manuela Gangemi* have also developed a practical and effective tissue-print test based on 13C5.**

Our polyclonal antiserum was not efficient at trapping psorosis virus particles by ISEM, but 13C5 can do it. Again, there has been good correlation between indexing, ELISA and typical particles seen in the EM, reinforcing the feeling that psorosis really is caused by this virus. (Of course some psorosis-like symptoms, not indexing as psorosis, may still be caused by different agents).

In another project with *Enrico Luisoni, Gian-Paolo Accotto, Ema Noris and Gabriella Rustici* from our lab, Indian citrus ringspot virus has been purified and partially sequenced, with probes and an antiserum produced, suitable for diagnostics (Rustici et al 2000, Archives of Virology, in press). This virus, first described as capillovirus-like, turns out to have affinities with potex-, carla, allexi- and foveaviruses, but does not fit any of these genera, so it may need to be housed in a new genus. (Though "looking" like a capillovirus, its genetic structure places it far away). The sequence has now been completed but is not yet written up.

Checking out the antiserum in India, we found (with *R.P. Pant* and *Y.S. Ahlawat* from IARI New Delhi) that the original isolate (K1, from Kinnow mandarin) was distinct from two other serologically related isolates, KK and K2, and further work may well uncover more family relations. An antiserum to K2 is available.

***Bob (Milne)* is now living in a very simpatico country farmhouse, probably over 200 years old and showing its age nicely. Wrinkles adjusted where necessary (there was no trace of bathroom or toilet, except out in the fresh air among the nettles). It's a bit cold for citrus in winter, but hopefully there will soon be a greenhouse. Please come visit, and do some gardening!**

MANI SKARIA - TEXAS

The microbudding project is receiving some recognition from the press and radio stations. Now, after being grown in the field since June 1997, it seems that not only the microbudded plants grow well in the field, but also it grew much better than I expected. Above all, several of the trees had fruit in less than two years after budding (including Rio Red grapefruit).

An international symposium on citriculture was held in Nagpur, India, November 23-27. I gave a talk on Microbudding for Biological Indexing and Commercial Tree Production.

MAHMOUD TAHER - FAO - EGYPT.

I spent most of the last two months in travel. My duty travel was mainly on Desert Locust problems and that took me to Jordan, Yemen and Rome. Apart from that, I traveled to the United Arab Emirates for a regional workshop on the problem of obsolete pesticides stocks in the Near East. I came back to base on the 1st of December. This will not be the last travel this year as I am taking off again on the 13th of January on a one week annual leave to Tripoli to visit my family.

THU HONG - VIETNAM

After recording the occurrence of seven virus and virus-like diseases (Huanglong bin and tristeza were the most important) in Vietnam citrus plantations, we implemented a Research and Development program with the support of CIRAD-FLHOR, ACIAR, FFTC and IOCV from 1995-1999. Now the program has reached a very successful output in terms of production of the disease-free mother plants. Sixteen citrus commercial cultivars with 107 selected clones and 28,708 seedlings have been produced up to now.

I am now chair of the two locally citrus projects of the two provinces in the Vietnam Mekong Delta and I

have completed my PhD thesis work titled: "Study on the Improvement of the aspects of plant protection for the citrus nursery" (Hanoi, Vietnam) the degree, however is not yet be gained as the thesis has been sent for final evaluation under the Ministry of Education and Training (the first evaluation under the Ministry of Agriculture and
Rural Development, Passed).

One of the sites of my internal project, was visited by *Patricia Barkley* and her group. She has made a report on this visit.

SHARON JONES - TRINIDAD-TOBAGO

With the indexing programme: We should complete our newly constructed psorosis laboratory next month. I have a plastic roofing on it and I hope that I get the desired temperatures within the room. I have on order an auxiliary air conditioner. I sent samples of our bark scaling type of disease to *Anna Maria d'Onghia* in Italy for analysis to see if the disease we see is psorosis. At first I sent leaf samples. The leaf samples were negative but I had also included some of the positive psorosis material Roistacher gave me i.e. P-205, P-208 and P-209 and the results were also negative. Then last year I sent some bud wood samples but as yet they have no results. In the field however I have not observed any increase in symptoms.

Tristeza deaths are still limited primarily to grapefruit. We are however monitoring for any sublethal effects of the disease in Valencia orange and grapefruit. Will let you know how that pans out as we are in the midst of collecting data for this season.

PEDRO MORENO - SPAIN

Greetings from the IVIA and my best wishes of a joyous and successful year 2000 for all friends in the IOCV. The past year was a busy one and 2000 likely will continue so. The cooperative research project on CTV and psorosis, funded by the European Union and developed in the laboratories of *Ricardo Flores* (IBMCP, Valencia, Spain), *Bob Milne* (IFA, Torino, Italy), *Oscar Grau* (IBBM, La Plata, Argentina), *Norma Costa* (INTA, Concordia, Argentina), *Marta Francis* (MGAP/INIA, Montevideo/Canelones, Uruguay), *Marcos Machado* (IAC, Cordeiropolis, Brazil) and in my lab, ended up on September 30, 1999. A coordination meeting to discuss the last pending aspects of the project was held on July 24th at La Plata, Argentina. This was a great opportunity for all project participants to get together and discuss future lines of research and cooperation. The meeting was synchronized with a two-weeks international course on *Graft-transmissible diseases of citrus and modern diagnostic techniques*, funded by CABBIO and organized by *Oscar Grau* at the Universidad de La Plata and Estación Experimental de INTA (Concordia). The course was attended by 18 Latin American students and lectured by several project participants. A most rewarding aspect of this visit was the possibility of enjoying the warm hospitality of *Laura Garcia* and *Oscar Grau.*

In our research on CTV we continued searching for genomic regions associated to the most severe isolates and trying to develop quick molecular procedures to identify these isolates and to monitor cross protection. A protocol for tissue print hybridization under stringent conditions developed in my lab is very convenient for massive testing, and at least two sets of probes seem to discriminate between severe isolates causing stem pitting on sweet orange and/or grapefruit, and isolates which are asymptomatic on these hosts. We also continued exploring variability of viral populations by SSCP analysis and sequence comparisons, distribution of these populations within infected plants and changes caused by graft or aphid transmission to a new host or by challenge inoculation with a different CTV isolate. I believe that modifications in the structure of the genomic and defective RNA populations induced by the above factors may explain most of the variability observed in pathogenic characteristics of CTV isolates.

The following three papers on these topics will be published soon:

***ALBIACH-MARTI, M.R., GUERRI, J., HERMOSO DE MENDOZA, A., LAIGRET, F., BALLESTER-OLMOS, J.F., MORENO, P.* (2000) Aphid transmission alters the genomic and defective RNA populations of citrus tristeza virus isolates. *Phytopathology* 90 (In press).**

***D'URSO, F., AYLLÓN, M.A., RUBIO, L., SAMBADE, A., HERMOSO DE MENDOZA, A., GUERRI, J., MORENO, P.* (2000). Contribution of uneven distribution of genomic RNA variants of citrus tristeza virus (CTV) in the plant to changes in the viral population following aphid transmission. Plant Pathology (In press).**

***NARVÁEZ, G., BEN SLIMANE, S., AYLLÓN, M.A., RUBIO, L., GUERRI, J., MORENO, P.* (2000). Hybridisation with digoxigenin-labelled cDNA probes to detect and differentiate isolates of citrus tristeza virus (CTV). Journal of Virological Methods (In press).**

Work on psorosis has continued trying to set up a quick and reliable diagnostic procedure under the Spanish conditions. We collected field isolates by selecting trees with different chlorotic leaf patterns and/or bark scaling symptoms from the most important citrus growing areas in Spain, put them in the greenhouse for symptom expression, and are testing them by ELISA with the monoclonals provided by *Bob Milne,* hybridisation, RT-PCR and EM. Correlation between psorosis diagnostic by symptom expression and by ELISA or PCR is fairly good. Particles characteristic of citrus psorosis virus (CPsV) were observed in a selection of these isolates. Isolates showing atypical symptoms are being examined for cross protection against psorosis B and for the presence or absence of CPsV particles. We expect to set up a protocol that will enable us to discriminate between leaf patterns caused by CPsV and those associated with other graft-transmissible diseases, particularly those of the previously called `psorosis group'. Sequencing of a Spanish psorosis isolate continues and we hope to end up in the following

months. We will also continue assessing variability of CPsV isolates from different areas, as this might limit detection of certain isolates, particularly by PCR procedures.

A cooperative work between *Pepe Guerri, Luis Navarro* and my lab is trying to characterize a new citrus virus, initially detected in kumquat but later found in other citrus cultivars in various countries. This is a filamentous virus which causes leaf blotching on Dweet tangor, and sometimes vein clearing in Pineapple sweet orange and incompatibility on citrange; however, it is not clear if the latter symptoms are caused by some variants of the same virus or our isolate contain more than one virus. The virus seems to be at low concentration and unevenly distributed in the infected plants, which is complicating development of quick diagnostic procedures. A first paper on biological characterization of this virus will soon appear:

GALIPIENSO, L., NAVARRO, L., BALLESTER-OLMOS, J.F., PINA, J.A., MORENO, P., GUERRI, J. **(2000). Host range and symptomatology of a graft-transmissible pathogen inducing bud union crease of citrus on trifoliate rootstocks. Plant Pathology (In press)**

RICHARD LEE - FLORIDA

I returned to Florida on July 1, 1999, completing my one year sabbatical leave with FAO. Tristeza and blight are still alive and well in Florida to keep me feeling needed here. I wish to remind IOCV members that the International Society of Citriculture Congress is being held in Orlando, FL from 3-7 December 2000. Information is available at: <http://www.lal.ufl.edu/isc_citrus_homepage.htm>

Also the Seventh Arab Congress of Plant Protection is being held from 22-26 October, 2000 at the University of Jordan, Amman, Jordan. Information is available at <tp://sacpp.ju.edu.jo> or from e-mail: Abstracts for presentation are due April 30, 2000.

DAVE GUMPF AND JOHN BASH - CALIFORNIA

THE CALIFORNIA CITRUS CLONAL PROTECTION PROGRAM IN TRANSITION

For over 40 years the Citrus Clonal Protection Program (CCPP) has played a vital role protecting California citrus from new diseases which could be introduced along with the importation of new varieties as well as eliminating the further dissemination of bud transmissible diseases already present in California. In the past, citrus cultivars important to California were "cleaned up" and established as Foundation budwood source trees in the CCPP Foundation Block located in the San Joaquin Valley at the UC Lindcove Research and Extension Center (LREC). Each year 15-20 new varieties are released from quarantine by CCPP. Many of these new releases have become leading commercial varieties in California.

The Foundation trees are routinely retested for all known bud transmissible diseases and maintained in healthy condition by appropriate care, periodic indexing for disease, yearly inspections of the trees and timely fruit evaluation. This information allows CCPP personnel to make valid assessments of individual budwood source trees in the Foundation Block as to trueness to type and freedom from abnormal characteristics. These field planted Foundation trees were registered with the California Department of Food and Agriculture (CDFA) following their guidelines for establishing a registered budwood source tree.

Recent actions by two of the five pest control districts in the San Joaquin Valley to no longer participate in the tristeza suppression program, have placed the field planted Foundation Block trees at an ever increasing risk of becoming infected with citrus tristeza virus (CTV). To insure that CCPP does not distribute infected budwood, Foundation trees are tested for CTV by ELISA each time budwood is cut for distribution (January, June and September). This is in addition to the normal practice of a yearly test on Mexican lime. This, however, is only a temporary solution because the decisions made by the pest control districts will most likely adversely impact our current practice of cutting budwood from the field trees in the not too distant future. For this reason, the citrus industry of California as represented by the Citrus Research Board, recommended that a large screenhouse be constructed at LREC to house the Foundation trees. The entire screenhouse facility will be constructed in stages. The initial 20,000 square feet of protected screenhouse has been constructed and all of the varieties represented by

the field grown Foundation trees have been propagated and are protected from infection in this screenhouse. At present all the trees in the screenhouse are maintained as container grown trees. As these container trees become larger, some will be transplanted to larger containers and some of the most popular varieties will be placed directly in the ground. Regardless of whether they are in containers or in the ground, when these protected trees attain sufficient size, they will be registered and used as budwood sources. By that time it will most likely be necessary for the CCPP to abandon its current practice of cutting from the field trees because of the danger of CTV distribution. As in the field collection, the numbers of registered trees of each variety will be adjusted based on budwood demand. Since the existing screenhouse is presently filled with container growing trees, there is a need to increase this protected space before we can begin to put any trees in the ground or transplant into larger containers. This additional screenhouse space will also provide the necessary space for additional propagations of the varieties with high budwood demand and for new varieties that are released (approximately 15 each year).

At the time budwood is cut only from the screenhouse trees, the present Foundation Block will be used as an Evaluation Block. On a routine basis, field trees will be propagated from the registered scion sources in the screenhouse and will be planted in this Evaluation Block so that growth performance and fruit quality may be evaluated. This is necessary to insure that budwood from our source trees results in the production of quality field trees producing fruit that is the highest quality and true to type. The future of CCPP will depend on a smooth and successful transition to these new procedures for variety maintenance.

ZHAO XUEYUAN - CHINA -

Zhao Xueyuan further reports: "The Chandler pummelo was introduced from the Lindcove foundation block in 1970 and about 100 thousand nursery trees have been produced and its propagation extended to some areas in China. In China, there are many superior local cultivars of pummelo. I suggest that Chandler will replace a part of them. The disadvantage of Chandler is that the fruit can not be stored as long as other cultivars such as Shatianyou".

An abstract on some interesting studies on the tolerance of the Chandler Pummelo to the citrus tristeza virus

Zhao Xueyuan, Zhou Changyong, Chen Quanyou, Yang Fangyun, Tang Keghi, Huang Sen, Jiang Yuanhui, Dai Shenggen, Li Taisheng, and Yang Xiubi (Citrus Research Institute CAAS Beibei, Chongqing 400712. 1999.
Chandler - A Preliminary Selected Pummelo Dwarf CTV) Tolerant or Resistant Cultivar of Pummelo. South China Fruit 1999;28(6):3-5 (Chinese with English summary)

Abstract-. Each of 4 budlings of 8 pummelo cultivars were graft inoculated with pummelo dwarf(CTV) in 1994. The observation conducted in 1994-1998 revealed that Chandler had not shown abnormal growth and only a few stem pits had been found. On the other hand, most budlings of the other 7 pummelo cultivars showed dwarfing, leaf curling and severe or moderate stem pitting. Chandler grafted on to diseased budlings or young trees in the field, grew without abnormality and without stem pitting. Chandler budlings graft-inoculated with pummelo dwarf collected from 4 localities also grew without abnormality and no stem pitting. The results of the above preliminary tests confirmed that Chandler is highly tolerant or resistant to pummelo dwarf.

Healthy

of Sick?

JOIN IOCV

LOUIS VON BROEMBSEN AND PAT NIVEN - SOUTH AFRICA

(This was submitted by Louis Von Broembsen and Pat Niven in July, 1999 - just missing the last IOCV Newsletter. It is presented here)

ROISTACHER TO RECEIVE HONORARY DEGREE

IOCV members will be proud to hear that the Council of the University of Pretoria, on the recommendation of the Senate, has decided to confer the degree D.Sc. (honoris causa) on Chester N. Roistacher. This degree will be conferred at a graduation ceremony to be held in Pretoria, South Africa, on 10 September 1999. The Council decided to honor Chester for his contribution to critical aspects of citriculture internationally and in particular for his role in establishing the principles upon which South Africa's highly successful Citrus Improvement Programme was based. South Africa's Citrus Improvement Programme is the foundation upon which the modern industry has been built. Its function has been to provide disease free, horticulturally selected certified propagation material to citrus nurseries and to run a nursery tree certification programme which has enabled citrus growers to acquire high quality disease-free trees for planting in commercial orchards. At the heart of this programme lies the elimination of viruses and other pathogens from the material entered into the scheme and the application of best operating practices in the production of citrus trees by commercial nurseries.

Developing technologies for eliminating harmful viruses from propagation material, determining accurate indexing techniques for determining the presence of certain pathogens and identifying the propagation practices, which most optimize the growth and productivity of new orchards, have been the life's work of Chester Roistacher.

His outstanding contribution to the Citrus Improvement Programme and the South African citrus industry as a whole has been made through numerous consulting visits and publications. It was felt that his efforts in bringing new technologies to this industry deserved special recognition. His association with the Plant Pathology Department of Pretoria University has been a long-standing and close one. Students trained in this department have made significant contributions in the application of the technologies pioneered or promoted by Chester Roistacher.

It is therefore appropriate that the University of Pretoria has chosen to honor Chet in this way. I'm sure that all who have experienced Chet's dedication and have had the privilege of working with him over the years will share our view that he greatly deserves the honor.

The University of Pretoria will host Chet for three days prior to the graduation ceremony after which he will undertake a series of lectures in various citrus production areas of South Africa to promote the concept of mandatory plant improvement schemes.

ANNA MARIA D'ONGHIA (ITALY)
AND KHALED DJELOUAH (ALGERIA)

We would like to give you some information concerning the main activities which we are carrying out on citrus through courses, cooperative projects and research.

<u>Courses:</u> Last October a new academic year of the international course on `IPM of Mediterranean fruit crops' started with the participation of 22 trainees coming from several countries (Albania, Algeria, China, Egypt, Jordan, Lebanon, Morocco, Palestine, Syria, Tunisia and Turkey). Among several lectures of the first part of the course, in December we had the contributions of *J.M. Bovè* and *M. Garnier*, who are deeply involved in teaching molecular biology techniques for the detection of different pathogens.

<u>Cooperative projects:</u> In the framework of the German-Egyptian cooperation project `Improvements of the citrus production in Egypt' the Mediterranean Agronomic Institute continued the consultancy, commissioned by GTZ, for the certification part of the project through two more visits, carried out in collaboration with the Egyptian Institutions.

***Field selection of 49 citrus candidate**
***Draft of a legislation for the mandatory control of CTV in Egypt**
***Organization of the Sanitation and Diagnostic Center**
***Project of an indexing greenhouse and a repository screenhouse**

GTZ also commissioned IAMB to do the disease assessment and indexing of selected citrus candidates and to do the short-term training of two technicians in graft-transmissible pathogen detection (serology and greenhouse indexing) and sanitation.

IAMB contributes to the project through:
***Long-term training. In particular a thesis on the sanitary assessment of citrus in Egypt started last October through the Master Program in IPM. Several samples have been collected and processed for the detection of the**

main graft-transmissible pathogens.

***ELISA testing for CTV and the citrus psorosis virus (CPsV) of the trees which will be used as budwood sources before the certification program will be operative.**

Within the cooperative project with the Ministry of Agriculture of Malta, the monitoring of CTV and CPsV and selection based on horticultural and sanitary qualities of citrus started in Malta last December. Preliminary results indicate no CTV infected trees were found and only 1.5% of the trees were CPsV positive.

The bilateral project on `Production, conservation and use of certified propagating material in Albania' is now continuing after some political problems. We are concerned with the selection and indexing of citrus for of 35 candidate selections. This activity will be carried out at IAM-Bari until structures will be established.

<u>RESEARCH:</u>
In collaboration with the Department of Plant Protection, University of Bari:

****Sanitary assessment of citrus in Palestine**
****Assessment by ELISA of CPsV transmission through seeds**
****Characterization of a new isometric virus in lemon**
****Standardization and validation of ELISA kits for the detection of the citrus psorosis virus.**

In collaboration with the Centro di Miglioramento Genetico degli Agrumi of Palermo, Italy:

**** Sanitation by somatic embryogenesis from style of different genotypes infected with the main citrus graft-transmissible pathogens (CTV, CPsV, CVV, CEVd and CCaVd). After three years from the embryogenesis the plants started to produce fruits under screenhouse conditions.**

Khaled DJELOUAH **completed his PhD in June 99, in the Plant Protection Department, University of Bari. His research was on the "Characterization of a Citrus psorosis virus -- local strains and the production of monoclonal antibodies against this virus". Also on the characterization of a new isometric virus in lemon. *G.M. Martelli, M.A. Castelano and V. Savino* were his co-major professors. He is presently collaborating on various projects in the Mediterranean Agronomic Institute of Bari (IAMB).**

BERNARD AUBERT - FRANCE

I was in Spain in November, 1999 at INIA, Valencia where a Mediterranean meeting was held on citrus. *Francis Llatser* told me that the Spanish citrus nurseries recently boomed to 7 million plants-- something quite unusual.

During December, I was on a trip to Haiti to establish a new programme of a citrus nursery on a private estate. From Haiti I went directly to Corsica where I met Rolland Cottin, **The director of SRA, Corsica who informed me that IPGRI-FAO has officially endorsed the new citrus description. This book will be printed in the coming weeks. it will be the official system for describing citrus varieties/cultivars.**

Finally, I am busy re-editing an old citrus book written nearly two centuries ago (see above)

My new address is B. Aubert - 28 Résidence Augusta - 101 Alleé de Port Pomant - 34380 La Grande Molle. FRANCE.

SUSAN HALBERT - FLORIDA

Florida Department of Agriculture and Consumer Services,

DIAPHORINA CITRI **- IN FLORIDA:**

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 colonisation? European Journal of Entomology 91: 121-125.

SEVERE CANKER IN FLORIDA

(From the New York Times, Jan. 17, 2000)

During the first two weeks in January, a serious outbreak of citrus canker occurred in southern Florida. It began showing up in backyards Florida, threatening the State's $8.5 billion citrus fruit industry. It has struck at least 20 commercial lime groves in Miami-Dade County, the hardest hit area. To try to check its advance, the State has quarantined nearly 700 square miles of land in and around the groves and has bulldozed and burned nearly 200,000 exposed and infected trees. The State and Federal governments have dispatched 125 inspectors into groves throughout South Florida.

The current infestation is concentrated south of Miami, around Homestead and Florida City. Today, state agriculture officials said they had inspected 581 of the area's 3,000 acres of commercial lime groves and found that 551 had been infected or exposed. All their trees will be destroyed. The canker has spread beyond Miami-Dade County; outbreaks have appeared in five other South Florida counties: Broward, Collier, Hendry, Hillsborough and Manatee.

The outbreak was traced to a home near Miami International Airport in 1995. Then the disease began appearing at other residential sites.

SALIBE - BRAZIL

I have not much to speak about myself. I am back to the Citrus Research Center where I started 44 years ago and where I worked with Citrus research for 12 years, before moving to the University.

My work now deals mainly citrus vein-enation/woody gall and tristeza viruses. Also doing some orange selection for lycopene content. Much writing, newsletter of the center and much information given to local newspapers. We are now starting to organize the 2001 Congress of the International Society of Citrus Nurserymen, planned for July, August or Sept. 2001. Much work, doing more now that I am retired than before. Warmest regards -- Ary A. Salibe

IOCV FINANCIAL STATEMENT

November 1, 1998 - October 29, 1999

CHECKING

1998 OCTOBER BALANCE: $19,126.49

1999 ENTRIES $2,156.66; $3,180.00; $103.31; $2,987.33

BALANCE ON OCTOBER 29, 1999 $23,240.47

***WALLACE FUND* 1998**

OCTOBER BALANCE $16,699.47

1999 ENTRIES $688.35

BALANCE ON OCTOBER 29, 1999 $17,387.82

SCWARTZ FUND

1998 OCTOBER BALANCE $2,674.93

1999 ENTRIES $110.14

BALANCE ON OCTOBER 29, 1999 $2,785.07

SAVINGS

1998 OCT. SAVINGS ACCOUNT BALANCE $608.11

1999 ENTREES $8.22

SAVINGS BALANCE OCTOBER 29, 1999 $616.33

1998 OCTOBER CD BALANCE $272.01

1999 ENTRIES $11.34

CD BALANCE ON OCTOBER 29, 1999 $283.35

TOTAL ASSETS, OCTOBER 29, 1999 $44,313.04

****

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)

NAME _____

ADDRESS _____

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COUNTRY _____

TELEPHONE _____;FAX_____

E-MAIL _____

MAIL $30.00 US (International money order or International draft PAYABLE TO: ***International Organization of Citrus Virologists***

Send to:

Chester N. Roistacher

Secretary, IOCV

Dept. Plant Pathology

University of California

Riverside, CA 92521-0122 USA

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		US$	US$	US$	US$	US$
7	___	15.00	3.00	18.00	_____	_____
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9	__	30.00	3.00	33.00	_____	_____
10	__	30.00	3.00	33.00	_____	_____
11	__	35.00	3.00	38.00	_____	_____
12	__	35.00	4.00	39.00	_____	_____
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