INTERNATIONAL ORGANIZATION OF CITRUS VIROLOGISTS

IOCV NEWSLETTER - SUPPLEMENT **April 13, 2006**

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ADDITIONS

Since publication of the hard copy of the newsletter (enclosed) additional information and pictures have come in after the announced deadline and will be published in this supplement.

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Short news from the group from Centro APTA Citros Sylvio Moreira, in Cordeirópolis, São Paulo, Brazil.

Functional Genome of *Xylella fastidiosa* and resistance of biofilm

Alessandra Alves de Souza – Centro APTA Citros Sylvio Moreira, Cordeirópolis/ SP, Brazil

Post genomics studies revealed that the pathogenicity of *X. fastidiosa* is a result of the biofilm formation inside the vessels leading to blockage and a consequent water stress. This is in contrast to the common pathogenicity mechanism, which relies on the activity of a type III secretion system, and therefore constitutes a new way of causing disease in plants. Comparative analysis of global gene expression of *X. fastidiosa* in biofilm and planctonic cells showed that several genes associated with resistance to antimicrobial compounds were up-regulated in biofilm cells even without these compounds. These results suggest that biofilm cells express constitutively some genes related with this resistance. The team of Centro APTA Citros Sylvio Moreira has evaluated the biological and molecular resistance of biofilm cells to heavy metals and antibiotics. Like bacteria that cause human diseases, the biofilm cells of *X. fastidiosa* were 2 to 10 times more resistant than planctonic cells. The highly expressed genes involved with resitance were evaluated by real time quantitative RT-PCR. Among the genes induced by the presence of the antimicrobial compounds with up to 10 fold induction are some that encode ABC transporters, proteins of the RND (resistance-nodulation-division) family, and others specific genes. Some genes showed high expression in presence of different compounds, indicating a cross action of the resistance mechanism. Although being preliminary, the study of *X. fastidiosa* biofilm resistance has showed that the control is more difficult when biofilms are formed. Nothing was known about the *X. fastidiosa* molecular mechanisms of resistance, for the reason this work open news perspectives to understand of biofilm dynamics of plant pathogens.

Transmission of the new variant of HLB Ca.*Liberibacter* to periwinkle by dodder (*Cuscuta racemosa*)

Helvécio Della Coletta Filho, Eduardo Fermino Carlos & Marco Aurélio Takita. Centro APTA Citros Sylvio Moreira, Cordeirópolis/ SP, Brazil.

HLB was first described in State of São Paulo, Brazil, in September, 2004, and nowadays one hundred municipalities have plants with HLB symptoms. Two genetic variants of bacterium associated to HLB were described in Brazil, *Ca. Liberibacter asiaticus* and *Ca. Liberibacter americanus*, being this last more broadly spread through orchards than the first one. In infected citrus plants, some characteristics of the bacterium associated to this disease are: low titer, uneven distribution in the plant, and a long period of incubation. Those characteristics impose significant difficulties on attempts to study the pathosystem. Therefore, an alternative host is needed, which may allow faster multiplication of *Liberibacter* to support other studies. We report successful transmission of the Ca. *Liberibacter americanu* from citrus to periwinkle by dodder (*Cuscuta racemosa*). Of the ten periwinkle plants inoculated by the dodder plant, seven have shown symptoms that could be associated to the presence of *Ca.* Liberibacter. The incubation period prior to first symptoms ranged from two to four months after the inoculation. The initial symptom on periwinkle was a localized yellowing in the leaves, after the entire leaves became yellow. Leaves on the same branch and from others branches also developed symptoms progressively, until the complete yellowing of the plant (Figure 1). Affected leaves of periwinkle were examined by PCR, with positive results for the American variant of bacteria. The presence of pleomorphic HLB associated bacteria in the affected plants also observed by electron microscopic (Figure 2).

Figure 1. HLB affected periwinkle showing generalized yellowing symptoms (Affected) beside a non-affected plant (Healthy).

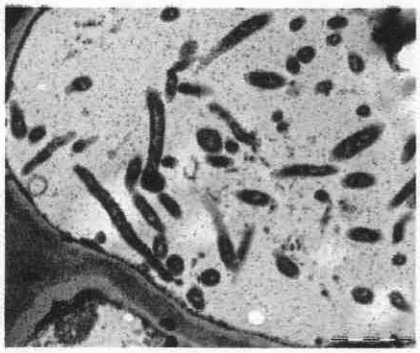

Figure 2. Ultrathin section from HLB affected periwinkle leaf showing pleomorphic HLB– associated bacteria (Picture by Francisco Tanaka).

What happened with citrus sudden death in Brazil?

Marcos A. Machado – Centro APTA Citros Sylvio Moreira, Cordeirópolis/ SP, Brazil.

Citrus sudden death (CSD) became a great concern in Brazil in 2001. From this time until now the anomaly was associated to over ??? million eradicated trees grafted on Rangpur lime. Since more than 85 % of the Brazilian citrus orchards (over than 160 million trees) are grafted on Rangpur lime, the destructive potential of CSD is enormous. CSD occurs only in the North of São Paulo State and Northeast of Minas Gerais. Although the number of trees is increasing within this area, new infected orchards outsides this area were not found so far. The problem seems to be concentrate within this area. Besides inarching with tolerant rootstocks (Cleopatra and Sunki mandarins, Swingle citrumelo), several growers are using irrigation to minimize damage caused by CSD. The etiology of CSD is still unclear. Both a marafivirus and CTV are candidates, but they are not consistently found associated in trees with typical symptoms of sudden death. Synergism between virus strains are not discarded as hypothesis. Our group developed a diagnostic of CTV based on RT-PCR of a typical genome strain found in plants with sudden death. Complete genome of CTV associated with sudden death trees was also finished. Two groups of CTV strains seem to be consistently associated in trees with sudden death, but one the this strain can also be found in healthy plants. What is now clear is that citrus sudden death has great influence of the climate conditions.

Combating Citrus HLB in São Paulo State, Brazil.

Eduardo Fermino Carlos - Centro APTA Citros Sylvio Moreira, Cordeirópolis/ SP, Brazil

After the first reports of HLB in São Paulo, Brazil, in 2004, the whole industry came to ask: "now what?" The disease is a worldwide well known threat to grow citrus wherever it happens, and reports from experts that served at infected places gave the appropriate concern of a serious problem. In the first half of 2005 legal support was create to locate, diagnosis and eliminate infected trees in São Paulo State. A 'task force' was assembled employing three organizations. Fundecitrus, funded by the industry to promote its sanitary defense, was in charge to inspect the entire São Paulo citrus belt. Once suspected plants were located, the State agricultural defense system was activated and their inspectors collected samples and sent them to us, at the 'Centro APTA Citrus Sylvio Moreira'. We have developed molecular (PCR) and visual diagnostic techniques that have allowed a massive diagnostic operation accessing to date 215 thousand trees, with 89.2% being positives (http://www.centrodecitricultura.br/). The numbers vary constantly. Prior characterizations of the bacterium distribution within the tree and research on type of leaf to be collected gave substantial accreditation to the whole system. Growers with positive trees were then notified by the State agricultural defense system and had limited time to remove all trees. To date, 75% of all positive trees have been removed, and the remaining ones are in process of. The campaign coordination was initially expecting reluctance from growers to endorse their own taking out trees, but the option was clear: "remove infected trees and have a chance to stay in business". By far, most of the growers have cooperated.

To further understand HLB, there are also different scientific researches being conducted in our lab: *Liberibacter* genome sequencing, search for plant responsive genes, field characterizations of symptoms, transmissions and epidemiological distributions. Other institutions have also collaborated extensively to the common cause. This project is founding by FAPESP.

The industry has no longer the expectation to completely eliminate HLB from our groves, in Brazil, but a sense of effective controlling over the disease has been established here more recently. In addition, previous practices such as vector control and production of healthy young trees under screened nursery system were inherited from citrus variegated chlorosis management and have proved effective also against HLB.

Workshop on Huanglongbing in Brazil

From July 17 to 20 will take place the International Workshop on HLB in Ribeirão Preto, São Paulo, Brazil. Several aspects of the disease in Brazil, Florida, China and South Africa will be presented. The organization committee includes Centro APTA Citros, Instituto Biologico, ESALQ/USP and Fundecitrus. More details in www.huanglongbing.com.br.

From: Bill Dawson - Florida

POSITION ANNOUNCEMENT #
Title: ASSISTANT PROFESSOR
MICROBIOLOGY AND CELL SCIENCE
Location: CITRUS RESEARCH AND EDUCATION CENTER
UNIVERSITY OF FLORIDA
INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES (IFAS)
700 EXPERIMENT STATION ROAD
LAKE ALFRED, FLORIDA 33850

Salary: **COMMENSURATE WITH QUALIFICATIONS AND EXPERIENCE**

Application Deadline: The position will remain open until filled; to assure consideration, applications should be received by the initial screening date of June 1, 2006.

DUTIES AND RESPONSIBILITIES: This is a 12-month tenure-accruing position at 90% research and 10% teaching located at the Citrus Research and Education Center at Lake Alfred. Tenure will accure in the Department of Microbiology and Cell Science centered at Gainesville. The research component is to focus on fastidious prokaryotic diseases of citrus, with particular emphasis on Huanglongbing (citrus greening). The faculty member will be expected to develop and maintain a strong, externally-funded research program, and will participate in graduate education, chair graduate committees and supervise thesis and dissertation research.

QUALIFICATIONS: A Ph.D. degree in Plant Pathology, Microbiology, or related field and a significant record of productivity as demonstrated through refereed publications is required. At least two years of postdoctoral research experience is desirable. Candidates with training in molecular plant-microbe interactions are especially encouraged to apply.

BACKGROUND INFORMATION: The Florida citrus industry is one of the largest fruit industries worldwide, and citrus is the most important agricultural crop in Florida (~800,000 acres). The Citrus Research and Education Center is an internationally recognized institution with research, extension, and teaching programs in biotechnology, horticulture, agricultural engineering, entomology and nematology, food science and citrus processing, fresh fruit handling, microbiology and cell science, plant pathology, physiology and biochemistry, post-harvest, precision agriculture, soil science, water management. The Center has a staff of approximately 200 people comprised of approximately 35 faculty members of the Institute of Food and Agricultural Sciences, University of Florida. The Citrus Research and Education Center (http://www.crec.ifas.ufl.edu) is located in central Florida, about 125 miles south of the University of Florida's main campus, 45 miles southwest of Orlando, and 50 miles east of Tampa. In the Lake Alfred vicinity are recreational facilities, modern schools, excellent health care, a 2-year community college, and two other public universities.

The home department will be the Department of Microbiology and Cell Science, which is an academic unit in the College of Agricultural and Life Sciences within the Institute of Food and Agricultural Sciences (IFAS) of the University of Florida (http://microcell.ufl.edu). It has well established and extramurally funded programs in the areas of immunology, microbial genetics, biochemistry, and physiology, as well as host-parasite relationships, innate immunity, and biotechnology. The Department offers B.S., M.S. and Ph.D. degrees through the College of Agricultural and Life Sciences; the undergraduate degree is also offered through the College of Liberal Arts and Sciences. Research programs are sponsored through the Florida Agricultural Experiment Station. The University of Florida, a land-grant university and member of the Association of American Universities, enrolls approximately 49,000 students in seventeen administrative units, including the Colleges of Agricultural and Life Sciences, Dentistry, Liberal Arts and Sciences, Medicine, and Veterinary Medicine, and the School of Natural Resources and Environment.

HOW TO APPLY: Applicants should submit a letter of application, a curriculum vitae, list of publications, copies of transcripts of all university work, and three (3) letters of recommendation by June 1, 2006. Nominations of candidates are encouraged. The names of nominees must be received at least one month before the closing date. Women and minorities are encouraged to apply. Please forward all nominations, applications and inquiries to:

REFER TO POSITION # __RETURN INQUIRES TO:

William O. Dawson
Chair, Search and Screen Committee
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850
Tel: (863) 956-1151; FAX: (863) 956-4631
E-Mail: wodtmv@crec.ifas.ufl.edu

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From Georgios Vidilakis - University of California
Junior Specialist for the Citrus Clonal Protection Program (CCPP)

CCPP is interested in hiring a Junior Specialist who is going to work with citrus plants and viral diseases of citrus as pan of the cooperative CDFA citrus tree registration program and the Citrus Clonal Protection Program.

The purpose of this position is to assist with the maintenance of greenhouse, screenhouse and field citrus plants necessary for the indexing of graft transmissible diseases of citrus. The Junior Specialist will also provide support to the CCPP laboratory in diagnostics and therapy of the virus and viroid diseases of citrus.

The candidate must hold a college degree or degree of higher education. Must be able to maintain flexible working schedule, including weekends. It will be required to make trips to Central California to contact field work. Must own a vehicle to commute between greenhouses and laboratory if needed. Must be able to perform duties in the outdoors and in the greenhouse in high temperatures or other weather conditions.

For more information contact Dr. Georgios Vidalakis at vidalg@ucr.edu or (951) 827 3763

Dr. W. P. (Bill) Bitters, eminent horticulturist died March 30th, 2006 at the age of 90. When quick decline (tristeza) began killing trees on sour orange rootstock, Dr. Bitters screened over 500 cultivars to determine their susceptibility to tristeza. It was this work that gave the Troyer and Carrizo citranges to the world as not only tristeza tolerant, but imparted excellent quality to the fruit.

In 1947, Dr. Bitters took over the world famous citrus variety collection at Riverside. He increased this collection from 600 to 1200 varieties and this collection is still recognized as one of the major collections of citrus genetic resources in the world. He was undoubtedly the world authority on citrus.

He will be missed!

Greening (HLB) in Florida

The following pictures of the greening disease in Florida taken by Susan Halbert and Tim Schubert were received after this newsletter was printed and are shown below to accompany Susan's article on pages 9 and 10. The article is repeated here with the pictures.

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Update on citrus greening (huanglongbing) in Florida

Susan Halbert and Wayne Dixon
Florida Department of Agriculture and Consumer Services, Division of Plant Industry

The Florida Department of Agriculture and Consumer Services (FDACS), with cooperation from the USDA, has been surveying for huanglongbing (HLB) since its discovery in the Miami area in August 2005. To date, we have found infected plants in twelve counties. The first delimiting survey indicated that infected residential citrus trees are common in the urban areas of southeast Florida (Miami-Dade, Broward, and Palm Beach Counties). We also have found infected plants in commercial citrus groves, but so far, HLB-infected areas are limited. A combined FDACS and USDA survey of approximately half of the square mile sections with commercial citrus in Southwest Florida resulted in positive finds in several groves; however, we did not find symptomatic plants in most of the sections we surveyed. Based on the surveys, State and Federal officials have determined that HLB cannot be eradicated in Florida.

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

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

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