VIIth ISSCT PATHOLOGY WORKSHOP

Louisiana, USA,
11-16 May, 2003

"ADVANCES AND CHALLENGES IN SUGARCANE PATHOLOGY"

Hosted by **ASSCT**
in collaboration with Louisiana State University Agricultural Centre and US Dept of Agriculture Sugarcane Research Unit
Louisiana, USA

- PROGRAMME
- **REPORT**

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The VIIth ISSCT pathology workshop was held in Louisiana (USA) from 11 to 16 May 2003 and was hosted by the American Society of Sugar Cane Technologists (ASSCT) in collaboration with the Louisiana State University (LSU) Agricultural Centre and the US Department of Agriculture Sugar Cane Research Unit. The theme of the workshop was "Advances and challenges in sugar cane pathology". The workshop was followed by a post workshop tour in Florida (USA) from 19 to 20 May 2003. The theme of the post workshop was "Florida pathology: research programmes and industry's disease concerns".

The workshop was attended by 37 delegates. Most of the world's leading sugar cane pathology scientists were present. Participants included 14 from the USA [Florida (4 delegates), Hawaii (2), Louisiana (8)] and 23 from outside USA [Argentina (1), Australia (2), Brazil (3), Colombia (1), Ecuador (1), Fiji (1), France (5), Guatemala (1), India (1), Mauritius (2), Nicaragua (1), Philippines (1), South Africa (2) and Thailand (1)].

1. Scientific presentations

A total of 24 verbal and 9 poster presentations were made under 5 session topics. Posters were displayed during the meeting and were also introduced by a short verbal presentation after the oral communications of each session. A discussion session was held after the verbal presentations of each session. Session topics and contributions were:

Sugar cane yellow leaf virus: 9 oral communications and 2 posters,
Other viruses: 4 oral communications,
Fungal diseases: 4 oral communications and 2 posters,
Bacterial diseases: 3 oral communications and 5 posters,
Sugar cane diseases: 4 oral communications.

1.1 Sugar cane yellow leaf virus (SCYLV)

This was the largest session devoted to a single sugar cane pathogen. Jack Comstock reported on the reliability of the leaf-midrib tissue blot immunoassay to detect Sugar cane yellow leaf virus and differences between cultivars in the rate of spread. Evidence was shown for a high number of false negative diagnostic assays in cultivar CP89-2143. Variable rate of disease spread between cultivars may reflect a type of resistance and will influence the effectiveness of using disease-free seedcane to control the disease. Jean Daugrois presented results from Guadeloupe showing that infection of sugar cane by SCYLV was associated with high populations of Melanaphis sacchari, one of the vectors of SCYLV. Jeff Hoy reported on an increase of SCYLV in Louisiana. Incidence of the virus is low in Louisiana and rates of spread seem to be lower than those observed in some other sugar cane growing regions. The reasons for this situation are still uncertain.

Current status of SCYLV infection in commercial cultivars in sugar cane growing regions worldwide was described by Axel Lehrer. Worldwide distribution of SCYLV was confirmed but SCYLV was not detected in samples from Morocco. Rapid spread of the virus was noted in several locations. Infection rate varied with sugar cane cultivars and age of cultivars.

Jeff Flynn reported on field trial evaluations comparing disease levels and yields in Kleentek® (tissue-cultured plants) and traditional seedcane sources among several varieties in South Florida. Fields established with Kleentek® seedcane generally showed the highest yields and yield increases of Kleentek® material over traditional seedcane (hotwater treated or not) were attributed to the impact of SCYLV. Yield reductions due to SCYLV in commercial production were also presented by Jorge Victoria, and varieties showing the highest yield decrease in Colombia were not always those showing the highest infection rate.

Jorge Victoria also reported on resistance of sugar cane to SCYLV in Colombian cultivars. No correlation was found between number of aphids (Melanaphis sacchari) per plant and virus incidence, nor between SCYLV particle concentration in plants and virus incidence. Cultivar CC85-92 showed outstanding resistance to both SCYLV and aphids. Jorge Victoria presented a third paper on transgenic plants of cultivar CC84-75 resistant to SCYLV. Several transgenic lines containing the coat protein gene of SCYLV were free of the virus after inoculation in the greenhouse using the aphid vector M. sacchari. Resistant plants are now to be tested in the field. Michael Davis also reported on transformation of sugar cane with the coat protein gene of SCYLV. Transgenic lines resistant to

the virus in the field were obtained with cultivar CP92-1666 but the transformation efficiency was low.

Axel Lehrer presented a poster on purification of SCYLV and antiserum production. Purified virus preparations were obtained from leaf and root tissues of sugar cane and injected to rabbits. An antiserum against Escherichia coli-expressed virus coat protein was also made. Cross reactions were sometimes observed between plant tissues and the antisera but not with purified IgG. Samples of antisera were distributed to several workshop delegates for further testing.

Philippe Rott presented a poster on genetic diversity of SCYLV isolates from several countries and especially from Réunion Island. Several genotypes were identified among a world collection of SCYLV isolates. The isolates were classified into two to four phylogenetic groups. Results suggested that SCYLV was introduced to Réunion Island from another country, and that one particular genotype evolved and spread on the island.

1.2 Other viruses

Three papers were presented on diversity of viruses causing mosaic. Jean-Claude Girard reported on the diversity of Sugarcane mosaic virus (SCMV) strains in central Africa. Sequence data suggested the isolates from Cameroon and Congo belonged to different phylogenetic groups. An inoculation experiment suggested that isolates from Congo were more pathogenic to R570 but this was not confirmed in a repeat experiment. There was some discussion on whether differences in aggressiveness in SCMV strains were observed in other countries. Some countries observed differences in variety reaction from that of other countries but the strain of the virus involved was not always known.

Mike Grisham reported on a survey of SCMV and SrMV (Sorghum mosaic virus) strains in Louisiana and how these have varied over time. SrMV strain I is now the dominant strain in Louisiana, with strain H being the next most common. Some collections did not match the known strains. Discussion centred on the identity of the new strains and that further work is in progress at USDA in Louisiana on identifying the new strains.

The incidence of the recently reported Sugarcane streak mosaic virus (SCSMV) in Asia was presented by Philippe Rott. A high percentage of samples showing mosaic symptoms from Bangladesh, India, Pakistan, Sri Lanka, Thailand and Vietnam were infected with SCSMV. SCSMV is a member of the Potviridae family but is not a member of the Potyvirus genus. Antiserum and PCR primers designed for potyviruses do not react with SCSMV. The consequences of this new virus to international movement of sugar cane germplasm was discussed. It was suggested that the ISSCT Pathology Committee should coordinate a project to make available antiserum that will detect all known viruses causing mosaic in sugar cane to increase the safe movement of germplasm.

New techniques for screening clones for resistance to Fiji leaf gall were presented by Barry Croft. The techniques were based on inoculation of 4-5 month old plants with infective Perkinsiella planthoppers in the greenhouses. The question whether Fiji leaf gall was spreading to new locations was raised but no one knew of any spread of the pathogen.

1.3 Fungal diseases

Salem Saumtally reported on the characterization of yellow spot (Mycovellosiella koepkei) and brown spot (Cercospora longipes) pathogens of sugar cane. Restriction digests of the Internal Transcribed Spacer (ITS) DNA region after PCR amplification did not reveal any polymorphism among 29 isolates of M. koepkei from Mauritius. Sequencing of this region confirmed that no variation was present. In contrast, the brown spot pathogen from Mauritius showed variability in symptoms induced on the same variety and cultural differences (colony morphology, pigment production, rate of growth) and pathogenicity. Genotypic variability was observed in the fungus after restriction enzyme analysis of the amplified ITS region.

Kushal Raj presented data on comparative performance of sugar cane genotypes to different pathotypes/isolates of the red rot pathogen. Different pathotypes of red rot (Colletotrichum falcatum) existed in different zones in India. Six pathotypes were used to inoculate 38 sugar cane genotypes. Eight genotypes had a differential reaction to the various pathotypes. The information obtained in this study will be useful to make the screening program more efficient and in the management of varieties in the different zones.

Kathy Braithwaite reported on genetic variation within a worldwide collection of sugar cane smut (Ustilago scitaminea) isolates. DNA from 38 smut isolates collected from Asia, Africa, South America, USA and Western Australia were prepared from single basidiospores. Genetic variation estimated from 12 AFLP primer combinations showed that overall there was little variation in the smut population across the world. However, isolates from Philippines, Taiwan and Thailand formed a distinct cluster. Susan Schenck reported on the differentiation of races of U. scitaminea in Hawaii. Observations conducted in Hawaii showed 20% smut infection in the resistant variety H 78-7750. A smut assessment trial with 10 main Hawaiian commercial varieties inoculated with the old isolate and the one from H 78-7750 indicated a marked change in the reaction of some of the varieties to the new isolate, possibly due to the appearance of a new race. A genetic study of smut isolates is being undertaken.

Barry Croft presented a poster on sugar cane smut incursion management in Australia. Quarantine regulations are being instituted to restrict smut to the north of Western Australia and prevent its spread to the east coast where the main sugar cane cultivation area of Australia (99% of production) is situated. Of the 700 clones being tested against smut in Indonesia, 70% have so far been found susceptible.

Barry Croft also presented a poster on the management of the orange rust epidemic in Australia. Orange rust (Puccinia kuehnii) is considered as the most damaging disease in Australia when it was previously considered as a minor disease. Germination of spores of the pathogen falls below 97% RH and temperatures above 30oC. Fungicides cyproconazole, tebuconazole, propiconazole and mancozeb were effective in controlling the disease, the best one being cyproconazole. Treated and untreated cane revealed losses between 11 to 29% tonnes sugar per hectare. A program is in place to replace the susceptible varieties as quickly as possible.

1.4 Bacterial diseases

Asha Dookun-Saumtally reported on the characterization of Sugarcane yellows phytoplasma (SCYP) from Mauritius. Based on RFLP analysis of the 16S rRNA operon of several isolates of SCYP from Mauritius, three distinct genetic groups were identified (SCYP1, 3 and 4). 16S rRNA fragments were cloned and sequenced and phylogenetic studies revealed that the SCYP1 phytoplasma was closely related to Stolbur phytoplasma whereas SCYP3 and 4 were associated with Coconut lethal yellowing phytoplasma.

Kathy Braithwaite presented the work of Young and others who reported on the genetic analysis of Australian and international isolates of Clavibacter (Leifsonia) xyli subsp. xyli. Two molecular biology techniques were employed to investigate the genetic diversity among an international collection of strains of the ratoon stunting pathogen. The techniques involved the use of PCR and the BOX primer set to produce DNA fingerprints of the strains, and the comparison of intergenic spacer (ITS) regions between rRNA genes by single-stranded DNA conformational polymorphism (SSCP) analysis. No variation was found among strains by either technique indicating that the pathogen was genetically uniform regardless of geographic origin. Additionally, these researchers reported that information obtained from the sequences of the ITS DNA was used to develop a highly specific PCR assay for detection of C. xyli subsp. xyli. The second paper on RSD presented by Jeff Hoy described the successful control of the disease in Louisiana through a public and private sector partnerships based on the use of RSD-free seedcane produced commercially and marketed under the product name Kleentek®. Repeated surveysfrom 1997 - 2002 indicated a decrease in the incidence of the disease that could be attributed primarily to the use of Kleentek®. Additionally, the control obtained with Kleentek® plants was more effective than the control obtained by heat treatment of seedcane.

Jean Daugrois reported on the aerial transmission of Xanthomonas albilineans in Guadeloupe. Strains of the leaf scald pathogen were found in free water on leaf surfaces early in the morning on sugar cane developing from disease-free tissue culture plants. Interestingly, in one study a change in the prevalent serotype of the pathogen was observed over time resulting in apparently more virulent serovar 1 strains replacing serovar 3 strains.

L.C. Assumpcão presented a poster on the comparison of the ITS regions of six different xanthomonad species pathogenic to sugar cane and found very restricted variability. The method was not able to differentiate the false red stripe xanthomonad from that causing gumming disease. However, in the poster presentation by A.C. Marchiori, rep-PCR was reported to produce unique fingerprints for all six pathogenic xanthomonad species. Furthermore, rep-PCR was used to look at the population structure of the false red stripe pathogen in Brazil and only one profile was found indicating uniformity within the pathogen.

Laurent Costet presented a poster on the genetic variability in xanthomonads pathogenic to sugar cane in Réunion Island and found RFLP analyses, using avr and hrp gene probes from other plant pathogenic xanthomonads, were useful for looking at variation among strains of the gumming disease pathogen Xanthomonas axonopodis pv. vasculorum, but not X. albilineans. Several haplotypes were observed among the gumming disease pathogen but this genetic diversity could not be correlated with pathogenic diversity among the strains examined.

C. Savario presented a poster on a study directed at elucidating factors associated with the "yield decline" in soils with a long-term sugar cane cropping history. Microbial communities varied between sites with a long-term sugar cane cropping history. Differences between the microbial communities were shown by culturing on different culture media and by examining the sole source carbon utilization profiles of the communities.

1.5 Sugar cane diseases

Barry Croft presented the results of a disease survey of the Nusa Tenggara province of Indonesia, the island bridge connecting Australia and New Guinea. Sugar cane smut and leaf scald were found on M442/51 on the island of Sumbawa. Mosaic-like symptoms were found on Sumbawa and Lombok islands but PCR-primers for mosaic did not detect the virus. Also present were ratoon stunting (one plant) and wide spread occurrence of orange rust, chlorotic streak and eye spot. Any new pathogens that are introduced to New Guinea will threaten the wild germplasm on the island and may eventually spread to Australia.

Jacqueline Ramallo presented the status of the current sugar cane disease situation in Tucumán, Argentina. Ratoon stunting is a major disease causing losses on CP 65-357. Brown rust is prevalent but has not caused any detectable losses. Other diseases that are observed but cause no losses are: red stripe, pokkah boeng, and brown stripe. Sugar cane yellow leaf virus has been detected. A disease-free seed program based on micro-propagation was initiated in 2000 to control ratoon stunting and other systemic pathogens.

Fe Dela Cueva presented the quarantine scheme initiated in the Philippines that is based on hot-water-treatment (24 hours pre-soak and 3 hours 50°C) and serological and polymerase chain reaction (PCR) assays. PCR assays are used to detect leaf scald, ratoon stunting, Sugarcane yellow leaf virus and Fiji leaf gall. ELISA is used to detect Sugarcane mosaic and sorghum mosaic viruses.

Eder Giglioli reported on the expressed sequence project (SUCEST) in Brazil. Over 250,000 expressed sequence tags (EST) were determined in the sugar cane genome. These ESTs are sites in the genome that are being copied to produce the required enzymes for metabolic activities (growth, disease resistance, etc.). The nucleotide sequences of these ESTs are being compared with the sequences of known genes in other plant species in DNA data banks to determine possible markers for disease resistance genes and/or other genes of interest.

2. Information and questions to the workshop participants

Besides the above mentioned sessions, several topics were discussed during the workshop.

2.1 General requests/information from ISSCT executive

Several questions from the ISSCT executive were submitted to the workshop delegates by the pathology section chairman. Discussions resulted in the following conclusions:

- "Biotechnology and its role in sugar cane research and production" could be a theme for one of the coming congresses. New initiatives related to future ISSCT Congresses: Suggestions included short oral introductions to poster presentations and maybe a discussion session; reduced cost for greater participation.
- No specific suggestion was raised regarding new initiatives that ISSCT could pursue to make it more relevant to international sugar.
- Possible improvements for the ISSCT web site: posting of
  * workshop abstracts,
  * data base (for growers and others) with sugar cane cultivars and their performance in each country (including disease resistance), * distribution of sugar cane diseases in the world (the list described in the Guide to sugar cane diseases should be updated).
- Pathology and breeding sections should hold a joint workshop in the future.
- Delegates agreed that if workshop presentations and abstracts can be gathered together on a CD for distribution to participants, no workshop proceedings should be published.
- Delegates questioned the permission requested by ISSCT from each author of a workshop paper to allow distribution of abstracts from workshops to trade journals; this permission can only be given by each individual and should not be a prerequisite to participate at a workshop. A similar rule should be applied to congress communications (full papers) that could be included in an ISSCT journal. Transfer of author copyrights to ISSCT of scientific communications might restrict the submission of good papers that could be submitted to scientific journals.

ISSCT report from the Executive was read by pathology section chair, Philippe Rott.

- Dates for next ISSCT Congress are:
  * January 28-30, 2005 - pre-Congress tour
  * January 30 - February 4, 2005 - ISSCT Congress
  * February 6-7, 2005 - post-Congress tour
- Location: Silver Jubilee Congress in Guatemala.
- ISSCT requests high quality papers and posters (about 40 slots in total for the four sections of the Biology commission).

2.2 Themes and locations for next pathology workshop

The possibility of a joint workshop with the molecular or breeding sections was discussed. Most participants favoured asking section chair, Philippe Rott, to contact the breeding workshop organizers about the possibility of having a joint workshop with them. The next workshop will probably be in late 2005 or early 2006.

Theme: Advances and challenges in sugar cane pathology.

Locations: Several organizations offered to host the next ISSCT pathology workshop:

- Cana Vialis in Brazil,
- CENICA in Colombia (with the possibility to host a joint pathology-breeding workshop),
- Cirad in Guadeloupe [+ post workshop in another Caribbean island (Barbados?,..)],
- Cirad in Réunion Island [+ post workshop in Mauritius? Or subsequent pathology and molecular biology workshops in Réunion Island and Mauritius, respectively],
- MSIRI in Mauritius (in case of a joint pathology-molecular biology workshop)

Potential hosts were instructed to prepare their invitations for presentation to the Executive at the next Congress.

2.3 Proposals for committee 2005 (January)-2007(August)

Current chair, Philippe Rott, was appointed to serve at least two terms and agreed to continue as chair through the next Congress. Current committee members have expressed a desire to continue to serve with the exception of Anusorn Kusalwong.

- members interested in remaining: Jack C. Comstock (USDA Florida/USA), Barry Croft (BSES/Australia), Philippe Rott (Cirad/France), Salem Saumtally (MSIRI/Mauritius).
- members intending to stand down: Anusorn Kusalwong (retirement from DOA/Thailand in 2005).
- members of ISSCT who expressed an interest in serving on the ISSCT Pathology Committee included Michael J. Davis (University of Florida/USA), Michel Grisham (USDA Louisiana/USA), Govind P. Rao (Sugar cane Research Station, Gorakhpur/India) and Jorge Victoria (CENICANA/Colombia). All agreed to serve if selected.
- member for nomination of section head: Philippe Rott.

2.4 Sugar cane Pathologists' Newsletter & Email list of sugar cane pathologists

Delegates agreed to revive the Sugar cane Pathologists' Newsletter (SPN) but an electronic version appeared to be the only practical format for this newsletter. It could be emailed to members and posted on the ISSCT web site if ISSCT agrees. SPN should contain any relevant information regarding sugar cane pathology (including a list of recent publications) and each contribution should be limited to one page. It should be published two times per year. The pathology committee members will serve as editors of SPN but will need the help of other members. SPN subcommittees will be identified in the near future.

2.5 Miscellaneous

- At the beginning of the workshop, the recommendations of the International Society of Plant Pathologists committee on Common Names of Plant Diseases were distributed to the workshop participants. An explanation of the reasons for the suggested name changes was presented by pathology section chair, Philippe Rott. The primary goal is to have a more informative name based primarily on symptoms that can be better understood among workers in different commodities. Comments on the suggested name changes were solicited from the workshop participants. Suggested new names are described in Annex 1 and further information can be found on the website at: www.isppweb.org

  The suggested new disease names were discussed and approved by all the workshop delegates.
- There is a need for a list of geographic disease distribution and/or official sugar cane disease and pathogen names on the web site of ISSCT;
- Several international collaborative projects were suggested such as resistance to red rot (Kushal Raj) and detection of viruses causing Sugarcane mosaic (polyvalent antiserum or universal PCR primers).
- A new name, *Sporisorium scitamineum*, was published to replace *Ustilago scitaminea* (Mycological Progress 1: 71-80, 2002) but this new name created controversy among several plant pathologists in the world. Future will tell if this new name will be used by plant pathologists, especially if *Ustilago* species from maize and other plants (barley,.) are also transferred to the new genus *Sporisorium*.
- A film presenting the next ISSCT congress to be held in Guatemala (February 2005) was presented during the workshop.

2.6 Brief up-dates on important disease problems and current research projects

The presence of delegates from different and various sugar cane producing locations was the opportunity to up-date information regarding disease situation and current research project in sugar cane pathology (see Annex II). Since the last workshop, only one report of a new disease discovery: ratoon stunting disease was found for the first time in Papua New Guinea.

3. Field trips

Two days were also devoted to field trips and the delegates visited the USDA Ardoyne research farm, the USDA Sugar cane Research unit in Houma, the Kleentek® seedcane farm, the Cameco research farm and the LSU AgCenter research farm at St. Gabriel.

4. Report on Post Workshop Tour

Ten participants from six countries attended the post workshop tour, which visited the Florida sugar cane industry and sugar cane research facilities. On May 19th, they visited the USDA-ARS Sugar cane Field Station, Canal Point, Florida reviewing the variety development and pathology programs and observed the screening programs for ratoon stunting, leaf scald and mosaic resistance. On May 20th the participants observed mosaic, eye spot, smut, and leaf scald in grower fields and visited research plots at the Everglades Research and Education Center, University of Florida IFAS, Belle Glade, Florida and the Research Department of the US Sugar Corporation, Clewiston, Florida and their sugar refinery.