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"Advances and challenges in Sugar Cane Pathology"

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Session 1: Pathogen variability

Genetic diversity within collections of the sugarcane orange rust fungus

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Orange rust, caused by Puccinia kuehnii, was once considered a minor pathogen in the Australian sugar industry. In 2000 it devastated the once top variety Q124, which represented 45% of the Australian crop, and caused the industry over A$150 million in yield losses. The industry resorted to controlling the disease with fungicides because of the lack of sufficient resistant planting material. Fortunately the level of resistance in the BSES breeding program is high and since 2000 new resistant varieties have been deployed to replace Q124. At the time of the epidemic very little was known about the genetic and pathogenic diversity of the fungus in Australia. It was believed that a new race developed in Australia or entered Australia from overseas. The aim of this project was to develop molecular methods to track changes in the pathogen and attempt to identify the source of the new race. As information on pathogen's biology was very limited, basic techniques for the storage and germination of spores had to be established. A detached leaf inoculation set-up was also established to generate pure single-spored isolates. Three ribosomal DNA regions were sequenced to assess the genetic diversity within orange rust from Australia, Papua New Guinea, Indonesia, China and historical herbarium collections. Limited genetic variation was detected within the Australian orange rust population suggesting that there is only a single dominant genotype present in the field. However, rust isolates from Indonesia and PNG, which appeared morphologically similar to orange rust, were genetically diverse. None of the Indonesian or PNG isolates tested appeared to be closely related to brown rust (Puccinia melanocephala). Phylogenetic clustering did not correlate with location or host. The results have revealed greater diversity in sugarcane rusts than previously thought. It is not yet known if these rusts could pose a quarantine threat to the Australian sugar industry.

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Analysis of Ustilago scitaminea genetic diversity using microsatellite markers provides evidence of selfing and dispersal of a unique lineage over America and Africa.

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Ustilago scitaminea Sydow, which causes sugarcane smut disease, has been spreading throughout Africa and America since the 1950s. The objectives of the present study were (1) to confirm and further describe the global population structure of U. scitaminea on the basis of a larger number of isolates and (2) to infer its reproduction system. Microsatellite markers, that present the advantage of being highly polymorphic, PCR-based, reproducible and codominant, were therefore developed for U. scitaminea and used to analyse a sample of single-teliospore isolates from various sugarcane-producing countries around the world. We surveyed 142 single-spore isolates of Ustilago scitaminea for genetic diversity. The fungal samples were teliospores from 77 single whips (sori) collected on various cultivars and at different locations in 15 sugarcane-growing countries throughout the world. The overall genetic structure of this fungus was investigated using 17 polymorphic microsattelite loci. All isolates but one were homozygous for all loci, indicating that selfing could be the highly preferential predominant reproductive mode of U. scitaminea. In America and Africa, genetic diversity was found to be extremely low and all isolates belonged to a single inbred lineage. This inbred lineage was also found in some parts of the Asian continent where most U. scitaminea genetic diversity was detected. These observations support the hypothesis that the fungus originated in Asia. The strong founder effect observed in the global genetic structure of U. scitaminea suggests that the fungus migrated from Asia to other continents on rare occasions.

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Variability of isolates of Ceratocystis paradoxa

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Pineapple disease, caused by the fungus Ceratocystis paradoxa (Dade) Moreau, is an important rot of sugarcane seed pieces or setts. The disease can cause considerable damage if conditions are favourable to disease development. Control of infection is achieved by dipping 3-budded setts in fungicides, which at times may fail. One of the factors that could be responsible for this situation is fungicide resistance. Variability in cultural characteristics, reaction to fungicides and pathogenicity of 18 isolates, previously collected from soils sampled from various regions of Mauritius (Y Moutia and S Saumtally, 1999), were studied. Growth rates of isolates on three media were variable (potato dextrose agar: 1.12 to 2.7 mm/hour, tomato juice medium: 0.5 to 2.69 mm/hour and Sabouraud dextrose agar: 3.00 to 7.35 mm/hour) as were their colony morphology (rings, star-like or uniform pattern; flat or cottony texture). Based on their growth rates and quantity of spores produced, eight isolates were chosen for pathogenicity tests on five sugarcane varieties (M 3035/66, M 1176/77, M 1394/84, R 570 and R 579). The aggressiveness of isolates did not differ significantly with respect to the position of the 3-budded sett on the same stalk. Cuttings taken from the bottom of the stalk were as infected as top ones. In contrast, the percentage of infection of cuttings incubated at temperatures varying between 20°C to 25°C and measured between day 8 and day 24 varied from 0% to 57.59%. Variety M 1176/77 was found to be relatively more resistant to all 8 isolates. The fungicidal effect of benomyl, difenoconazole, difenoconazole + carbendazim, hexaconazole, tebuconazole, thiophanate-methyl, trifloxistrobine was evaluated on ten isolates in vitro. The effective dose of difenoconazole + carbendazim that inhibited growth of colony size by 50% (ED50) varied from 0.070 to 0.086 ppm, whereas that for the commonly used thiophanate-methyl varied from 0.430 to 2.462 ppm. Fungicides benomyl, difenoconazole and tebuconazole also showed a high inhibitory effect on the fungus.

Keywords: Ceratocystis paradoxa, pineapple sett rot, sugarcane, cultural characteristics, fungicides, pathogen aggressiveness

Reference: Y Moutia and S Saumtally (1999), Detection from soil and distribution of Ceratocystis paradoxa Moreau, causal agent of the pineapple disease of sugarcane. Fourth annual Meeting of Agricultural Scientists, Reduit, Mauritius, p 75-81.

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Molecular diagnosis and genetic diversity of the causal agents of mosaic and streak mosaic of sugarcane

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Sugarcane mosaic is caused by the Sugarcane mosaic virus (SCMV) and the Sorghum mosaic virus (SrMV), and these two viruses belong to the genus Potyvirus and to the family Potyviridae. Sugarcane streak mosaic is caused by the Sugarcane streak mosaic virus (SCSMV), an unclassified member of the same virus family. Several strains have been described for each of these viruses, and several reverse-transcription polymerase chain reaction (RT-PCR) protocols were developed for molecular diagnosis of SCMV, SrMV and SCSMV. These protocols are generally based on the virus coat protein sequence. Because this sequence is often variable, the effectiveness of the RT-PCR assays to diagnose all isolates/strains of the pathogen needs to be tested. We therefore applied several RT-PCR assays to a collection of isolates of SCMV, SrMV and SCSMV.

The protocol of Yang and Mirkov (1997), modified by Alegria and co-workers (2003), allowed us to detect SCMV strains A, B, D and E, but also strains from Africa not yet described. This protocol failed, however, to detect SCMV in sugarcane from China showing mosaic symptoms. A phylogenetic study of sugarcane and maize isolates of SCMV by Chen and co-workers (2002) previously showed that isolates from sugarcane from China are genetically closer to Chinese and European SCMV isolates from maize than to SCMV isolates from sugarcane in Australia, South Africa and the USA. Additionally, we performed a comparison of published sequences of SCMV which showed that RT-PCR primers of Yang and Mirkov (1997) are not efficient for diagnosis of SCMV isolates from maize. Primers oligo1n and oligo2n for potyviruses of Poaceae developed by Marie-Jeanne and co-workers (2000) allowed us, however, to detect all strains of SCMV, including the SCMV isolates from China not previously detected. Primers oligo1n and oligo2n also proved to be very efficient in the diagnosis of SrMV. These primers do not distinguish between SCMV and SrMV, but are very useful for simultaneous detection of SCMV and SrMV in diseased plants, especially in sugarcane quarantine. If needed, virus species can be identified by sequencing the RT-PCR amplicon and determining sequence identity with known and sequenced viruses. Alternatively, restriction analysis can be performed. Primers cited above were not efficient in detecting SCSMV by RT-PCR in diseased sugarcane. In contrast, the protocol developed by Chatenet and co-workers (2005) using primers ST2-ST5 proved to be

efficient in amplifying a specific fragment from 32 sugarcane leaf samples showing streak mosaic symptoms. Amplified fragments were cloned and sequenced, and phylogenetic analysis confirmed that several strains exist within this virus species in Asia.

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Genotyping of Sugarcane yellow leaf virus in Colombia, Guadeloupe and Reunion

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Recent genetic diversity studies suggested the existence of several genotypes of Sugarcane yellow leaf virus (SCYLV), the causal agent of yellow leaf of sugarcane. In order to identify and more precisely describe the genotypes of SCYLV, we sequenced the entire genome (six ORFs) of eight virus isolates from six geographical locations (Brazil, China, Colombia, Cuba, Peru, and Reunion). Four genotypes of SCYLV (BRA for Brazil, CUB for Cuba, PER for Peru and REU for Reunion) were identified based on phylogenetic analyses with the genome sequence of these eight isolates. Specific primer pairs were designed to identify the SCYLV genotypes by RT-PCR. A unique genome fragment was amplified from each genotype, with the exception of genotypes BRA and PER which are relatively close. The RT-PCR primers were then used to identify genotypes of SCYLV present in three different sugarcane growing locations: Colombia, Guadeloupe and Reunion. Out of 41 sugarcane leaf samples from Colombia, 15 were infected by genotype BRA/PER, 19 by genotype CUB and 7 by genotypes BRA/PER and CUB. Both genotypes were found in locally bred CC varieties and in foreign varieties, but incidence of genotype CUB (84%) was higher than incidence of genotype BRA/PER (44%) in the 10 CC varieties. Out of 64 samples from Guadeloupe, 12 were infected by genotype BRA/PER, 13 by genotype CUB, 37 by genotype REU and 2 by genotypes CUB and REU. The three genotypes were found in locally bred FR varieties and in foreign varieties. In the 15 FR varieties, incidence of genotypes BRA/PER, CUB and REU was 16%, 37% and 47%, respectively. Out of 51 samples from Reunion, 2 were infected by genotype BRA/PER, 29 by genotype REU and 20 by genotypes BRA/PER and REU. Only genotype REU was found in the 29 samples from the 4 locally bred R varieties. All samples infected by genotypes BRA/PER and REU (or only BRA/PER) were obtained from variety SP-716163. Several genotypes of SCYLV can therefore co-exist in a geographical location or within a plant. Genotypes REU and CUB were, however, not found in Colombia and Reunion, respectively. Genotypes BRA/PER and REU are both present in Reunion, but genotype BRA/PER (most likely imported from Brazil with variety SP71-6163 in 1987) has not, as of yet, spread on this island. Because sugarcane is the only known natural host of SCYLV, and because this plant species did not originate from Colombia, Guadeloupe or Reunion, the virus was imported into these locations from other contaminated sugarcane growing locations. The presence of several genotypes of SCYLV suggests different virus introductions and/or an evolution of the virus after its introduction into a new environment. The biological significance of these SCYLV genotypes remains to be determined.

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Towards identification of genes involved in pathogenicity of Xanthomonas albilineans, the sugarcane leaf scald pathogen

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Numerous genes involved or putatively involved in pathogenicity of plant pathogenic bacteria have been identified. These genes code for different secretion system constitutive proteins, exopolysaccharides, virulence factors, toxins, plant cell-wall degrading enzymes, cell mobility and motility factors or adhesion factors. In contrast to most plant bacterial pathogens, no hrp or avr genes were found in Xanthomonas albilineans, the causal agent of sugarcane leaf scald. This pathogen produces, however, a pathotoxin called albicidin that is responsible for foliar disease symptoms. Recently, all genes involved in albicidin biosynthesis of strain Xa23R1 from Florida were cloned and sequenced. Variation in albicidin biosynthetic genes was, however, not correlated with variation in pathogenicity of X. albilineans. In this study, we attempted to identify new genes involved in pathogenicity of X. albilineans using several approaches and 19 strains of the pathogen differing in disease severity and stalk colonization in Guadeloupe. The in vitro production of albicidin varied among strains of X. albilineans, but all strains showed the same RFLP (restriction fragment length polymorphism) pattern with albicidin biosynthetic genes. Similarly, no variation was found among strains by PFGE (pulse-field gel electrophoresis). In contrast, variation among strains was found by AFLP (amplified fragment length polymorphism) with 16 selective primer combinations, after enzymatic digestion of total genomic DNA with SacI and MspI. No relationship between this genetic variation and variation in pathogenicity was, however, identified. A total of 40 primer sets were then designed to amplify by PCR (polymerase chain reaction) 40 genes involved in pathogenicity of bacterial species closely related to X. albilineans, and particularly X. campestris pv. campestris. Only one gene, pilB, could be amplified from total genomic DNA of nine strains of X. albilineans differing in disease severity and stalk colonization in Guadeloupe. Nucleotide sequence identity was 100% identical among the strains of the pathogen and a phylogenetic study with this sequence confirmed that X. albilineans belongs to the genus Xanthomonas. Absence of amplification with 39 primer sets suggested that genes involved in pathogenicity of X. albilineans differ significantly from those of other closely related pathogens. Sequencing of the whole genome of X. albilineans will be a great step in unraveling pathogenicity of this sugarcane pathogen.

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Session 2: Disease diagnosis and new diseases

The sugarcane quarantine of CIRAD in Montpellier, France: from disease diagnosis to fundamental research and vice versa

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Accurate disease diagnosis tools are a major need for a plant quarantine process. Thanks to the development of serological and molecular biological techniques, significant improvements were obtained in disease control in sugarcane quarantine, although "old traditional" methods such as isolation on selective culture media also remain very useful (for instance for the detection of Xanthomonas albilineans, the sugarcane leaf scald pathogen). Most of the time, new techniques can be rather easily adapted to the specific needs of disease diagnosis in quarantine; however, specific research activities must be undertaken i/ when a new disease appears and no diagnostic tools are available, and ii// to make sure that all strains or variants of a pathogen can be detected. For the above reasons, as well as for routine disease testing, CIRAD's sugarcane quarantine has been involved in research activities regarding the characterisation of emerging diseases and in genetic diversity studies of several plant pathogens. Within the last decade, CIRAD's sugarcane quarantine had to face two emerging diseases, yellow leaf, caused by the Sugarcane yellow leaf virus (SCYLV), and streak mosaic, caused by the Sugarcane streak mosaic virus (SCSMV). Tissue culture methods were used to eliminate SCYLV and SCSMV from infected varieties in quarantine, and studies on genetic diversity and variability in pathogenicity showed that these viruses were not homogeneous pathogens. Additionally, studies on SCYLV disease progress in the field and impact of yellow leaf on yields were carried out in Reunion Island where an unusual lineage of the virus occurs. Mosaic is another important disease to be detected in sugarcane quarantine. The disease occurs in more than 70 countries and the causal agents of mosaic, the Sugarcane mosaic virus (SCMV) and the Sorghum mosaic virus (SrMV), have been known and studied for several decades. Additionally, several molecular assays were developed for diagnosis and detection of these two viruses. Numerous strains have been described for SCMV based on plant inoculation and serological data, but most genetic diversity studies included only strains from Australia and the USA. We therefore studied the genetic diversity of a collection of SCMV isolates from eight countries, and especially 50 isolates from Africa. Our results showed that SCMV isolates are distributed into two major phylogenetic groups (sugarcane group and maize group) and several subgroups that are closely related to the country of origin of the isolates. Additionally, all isolates from sugarcane belonged to the sugarcane group. More recent discoveries revealed, however, that Chinese SCMV isolates from sugarcane belong to the maize group. These latter isolates were not detected by most molecular assays developed for the detection of SCMV from sugarcane. Fortunately, a PCR-assay developed for diagnosis of Poaceae viruses proved to be very efficient in the diagnosis of sugarcane and maize isolates of SCMV. This assay is also efficient to simultaneously detect SCMV and SrMV.

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Standardization of reverse transcription polymerase chain reaction (RT-PCR) for diagnosis of the Streak mosaic virus of sugarcane in Colombia.

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The disease caused by streak mosaic virus of sugar cane (SCSMV) is considered exotic in Colombia. Its incidence is considered up to 100% in India and other countries of Asia. CENICAÑA constantly is introducing sugarcane varieties of different origins, presenting the risk of introducing this or other pathogens to Colombia, therefore concerns the importance of standardizing the technique of RT-PCR as a method of diagnosis to detect the virus responsible of the streak mosaic of sugarcane, to be able to apply it in tests with the new introduced varieties and the ones located in the germbank. For the standardization of the diagnosis method, ARN extracted from plants with SCSMV supplied by Dr. P. Rott of CIRAD of France was used. The sequences of specific primers for SCSMV STR2 (forward), STR5 (reverse) and P1 (reverse) were also kindly given by Dr. P. Rott and were used in Rneasy Plant and RT-PCR kits of QIAGEN of a single step. Several temperatures of hybridization of the primers were evaluated (50°C-60°C) being that the optimal temperature of hybridization was 56°C, for primers STR2-STR5, producing the fragment of 400 pb and 50°C for primers STR2-P1, producing the 500 pb band, reported by Rott and others. All the results were visualized in a gel of 1.5% agarose in a transiluminador of ultraviolet light. Using the methodology standardized for the SCSMV and the technique of RT-PCR standardized previously for the virus of the common mosaic of the sugar cane (SCMV), twelve varieties of sugar cane were evaluated with symptoms similar to common mosaic virus of the germbank of CENICAÑA. Out of the twelve, 58% of the varieties were positive to SCSMV, 75% were positive to the common mosaic (SCMV) and 33% were positive to both pathogens. Leaves were taken from the 12 varieties with mosaic to inoculate mechanically healthy plants of sorghum Rio and sweet corn. Weekly evaluations of symptoms in the inoculated plants were made and only mosaic was found in those plants were SCMV was previously registered. The varieties that were positive solely to SCSMV did not express visible symptoms of mosaic in sorghum and corn plants. The standardized diagnosis method will be used to evaluate other varieties of the sugarcane germbank with symptoms similar to mosaic.

Keywords: Sugarcane streak mosaic virus (SCSMV), molecular diagnosis.

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Mosaic of sugarcane in Indonesia, China, Papua New Guinea and Australia.

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Disease surveys have been conducted in Indonesia, Papua New Guinea and Australia to determine the distribution of diseases that could threaten the sugar industries in these countries and other Saccharum species in the centres of diversity. In addition, samples of mosaic have been collected from Yunnan and Guangzhou provinces of China during visits to these regions, and samples from Thailand have been intercepted in Indonesia. Mosaic was found at a number of locations in Indonesia in both commercial and garden canes. Screening of these samples with ELISA for potyvirus and RT-PCR for sugarcane mosaic virus (SCMV), sorghum mosaic (SrMV) and sugarcane streak mosaic (SCSMV) revealed that all samples from commercial fields in Indonesia (Java) were

infected with SCSMV, while samples from germplasm collections were infected with SCMV and SCSMV, including mixed infections. SCMV was recorded in two samples from semi-commercial plots of noble canes on the island of Lombok but one sample showing classic mosaic symptoms from the island of Sumbawa did not react with assays for SCMV. Two samples taken from cane grown from setts from Thailand that were intercepted by the Indonesian quarantine authorities were infected with SCSMV. A historical collection of mosaic isolates maintained for many years by the Indonesian Sugar Research Institute contained isolates recorded as strains A, B and E of SCMV. Samples from clones reported to be infected with strains A, B and one sample of strain E tested positive for SCMV but one clone reported to be infected with strain E tested positive for SCSMV. None of the assays were able to detect a causal agent of mosaic in sugarcane showing excellent mosaic symptoms collected from East New Britain, Papua New Guinea. Investigations are continuing into the cause of these symptoms. Samples of mosaic collected from Yunnan and Guangzhou provinces in China were found to be infected with SrMV. In Australia only SCMV has been recorded. Mosaic is widespread in many Asian countries and although it does not cause dramatic yield loss, the potential for losses is great because of the high incidence of the disease. The need for a range of diagnostic assays to detect all known causes of mosaic and the existence of some mosaic symptoms that appear to be caused by as yet unknown agents means that all quarantine facilities need to work together and share their experience with diagnostic assays to prevent the spread of these viruses around the world.

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Antibody to a short peptide sequence detected Sugarcane yellow leaf virus isolates from several sources

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Sugarcane Yellow Leaf Virus (SCYLV) infects many sugarcane cultivars in sugarcane-growing areas around the world. Infected plants are often symptomless and diagnosis depends on PCR analysis or on one of several immunology techniques which require the use of a specific antibody. Although it has been done successfully, purification of SCYLV from infected plants is difficult because the virus is restricted to phloem cells and exists in low titre. Slight amounts of sugarcane proteins are likely to remain which may interfere with specificity of an antibody. Therefore, a project was undertaken that made use of published information on other Luteovirus coat protein structures, estimates of possible antigenic epitopes, sequencing of the capsid protein gene from a Hawaiian isolate and testing of antibodies to them for detection and specificity to SCYLV. The result was the production of one antibody that had the desired activity and specificity to be used for diagnosis of various isolates of SCYLV without interfering reactions to sugarcane tissue or to other related Luteoviruses or Poleroviruses.

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Evidence for a virus in Ramu stunt infected sugarcane

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A serious disease appeared at Ramu Sugar Limited in Papua New Guinea during the 1986 season and became known as Ramu stunt. It is characterised by a marked reduction in growth rate, seen as reduced internode length, reduced root system and failure to ratoon. Leaves are shortened, stiff and erect and can show striping, chlorosis and premature senescence. The symptoms vary depending on the cultivar. Spread between plants is rapid and the sugarcane leafhopper Eumetopina flavipes is the suspected vector. The causal agent is suspected to be a virus1 or a phytoplasma2. In 2000 BSES Limited received funding from the Australian Centre for International Agricultural Research (ACIAR) to assess pest and disease threats to Saccharum germplasm and sugar production in Papua New Guinea, Indonesia and Australia. Research into the causal agent of Ramu stunt is an important component of this project because of its severe impact at Ramu Sugar, the risks associated with moving quarantine cane from PNG to other countries and the risks to the Australian sugar industry posed by an accidental introduction of the leafhopper Eumetopina into the northern parts of Australia. Nested-PCR screening has so far failed to show the presence of any phytoplasmas in Ramu stunt infected sugarcane using the methods described in Suma and Jones3. This may be due to the condition of the leaf material received from Ramu due to the distance involved and the quarantine process in Australia. In contrast, we have obtained some preliminary evidence for a virus in Ramu stunt infected sugarcane. A viral miniprep method based on ultracentrifuging through two sucrose cushions has been developed. Small isometric viral particles have been observed under the electron microscope in minipreps extracted from the variety Ragnar. A characteristic 36kDa protein has been detected in viral minipreps extracted from seven Ramu stunt infected canes. The protein is not detected in healthy cane from PNG, or sugarcane infected with Fiji leaf gall virus or sugarcane mosaic virus. The protein has been subjected to peptide mass fingerprinting and internal sequencing. In both cases the protein did not significantly match anything in databases. A cloning approach is now underway in an attempt to identify disease associated nucleic acids.

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Detection and molecular characterization of phytoplasmas in sugar cane and weeds in Mauritius

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Sugarcane yellows is caused by sugarcane yellows phytoplasma (SCYP). It has been suggested that weeds growing in and around sugar cane fields may act as alternate hosts for the pathogen. In the present study, the polymerase chain reaction was used to assess the presence of the phytoplasma in both sugar cane and weeds in Mauritius. DNA was extracted from sugar cane and weed samples using the CTAB method. A nested-PCR approach was used to amplify a fragment of the 16S rDNA of the phytoplasma using universal primer pairs P1/P7 and R16F2n/R16R2. The expected 1250 bp fragment was amplified from sugar cane as well as weed samples including Cynodon dactylon, Sorghum verticilliflorum, and Eleusine indica. Restriction enzymes Rsa1 and Taq1 were used to determine the identity of the phytoplasmas present. The restriction profiles observed from phytoplasmas amplified from sugar cane varieties B 6504, B 50112 and SP716163 were different from SCYP groups previously described locally. For the phytoplasmas amplified from weeds, the restriction profile for Eleusine indica was similar to the new profile observed in sugar cane while the others were different suggesting diversity amongst phytoplasmas present both in sugar cane and weeds in Mauritius.

Keywords: phytoplasma, sugar cane, weed, sugarcane yellows, nested-PCR, RFLP

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Session 3: Disease impact on the sugarcane crop

Ratoon stunt and yellow leaf: Effects on sugarcane yields

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Separate trials were established to determine the effects of ratoon stunt, caused by Liefsonia xyli subsp. xyli (Lxx) and yellow leaf, caused by Sugarcane Yellow Leaf Virus (SCYLV), on sugarcane yields in plant and first-ratoon crops. In 1-m long plots, responses of eight cultivars were tested for ratoon stunt, and five cultivars were tested for SCYLV. For ratoon stunt, two trials compared the yields of healthy plants to Lxx-inoculated plants and one trial compared healthy to verified Lxx-infected plants. In the yellow leaf trials healthy and SCYLV-infected plants were compared. In each of the ratoon stunt trials, combining the data of all the cultivars (CP 70-1133, CP 72-1210, CP 72-2086, CP 80-1743, CP 80-1827, CP 84-1198, CP 85-1491, and CP 89-2143) yields were significantly reduced ranging from 3.5 to 15.8 % loss of sugar per plot. Statistically significant losses were not obtained for each cultivar in the individual trials; however, the infected plots consistently had lower yields with only a rare exception. For SCYLV, plot weight and kg sugar per plot for all cultivars (CP 72-1210, CP 80-1827, CP 84-1198, CP 85-1382 and CP 89-2143) combined were higher for healthy than for SCYLV-infected. Again statistical differences were not always significant in the yields of individual cultivars although the majority of yield parameters were higher in the SCYLV-free plots. The disease incidence must be taken into consideration when extrapolating yield trial results to crop yield. In Florida, the incidence of ratoon stunt varies between zero and 100 % in commercial fields but is not usually extremely high. Since the incidence of SCYLV is usually 85 % or higher in commercial fields, total commercial losses due to yellow leaf in Florida are probably higher than ratoon stunt.

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Relationship between extent of colonization by Leifsonia xyli subsp. xyli and yield loss in six South African sugarcane varieties

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The reaction of commercially grown South African varieties to ratoon stunting disease (RSD), caused by the bacterium Leifsonia xyli subsp xyli, has traditionally been based on large, replicated yield loss trials grown over a number of years under rainfed and irrigated conditions. These trials have provided valuable information on the effect of L. xyli subsp xyli on the varieties, but they are time-consuming and require large areas of uniform land. They are therefore not suitable for evaluating large numbers of sugarcane varieties during the variety selection process. A tissue blot-enzyme immunoassay (TBIA) that measures the percentage of colonised vascular bundles (%cvb) in RSD-infected sugarcane stalks has successfully been incorporated into the disease-screening programme at the USDA - ARS Sugarcane Field Station in Florida. Before introducing the method in South Africa, a trial comparing TBIA and yield loss as methods of variety evaluation was established. A good correlation between yield loss and %cvb in six commercial varieties was obtained from the results of a rain fed field trial conducted at Mount Edgecombe. The results from this study indicated that the TBIA could provide an effective and more efficient method for evaluating varieties for their reaction to RSD before their release to the growers.

Keywords: RSD, ratoon stunting disease, sugarcane, disease screening, TBIA, tissue blots

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Sporadic incidence of new disease syndrome of GSD and SCYLV causing severe losses to sugarcane crops in Eastern Uttar Pradesh, India.

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During survey of sugarcane crops of Maharajganj, Gorakhpur, Kushinagar, Basti and Siddharthnagar in May- August, 2005 unspecific yellowing and stunting of sugarcane clumps were recorded in many fields of variety CoSe 93232, 92423, 98231, CoLk 8102 and UP 9530 in all the abovementioned districts. No further growth of the affected crop was recorded and side shooting was also noticed in these clumps in fields of affected sugarcane var. CoSe 92423 and 93232 in Siswa Bazar, Maharajganj districts of Eastern Uttar Pradesh. In many fields, unspecific stunting showing symptomatic plants were investigated for the association of any virus/ bacteria/ phytoplasmas using IC-RT-PCR and Nested PCR assays. Association of GSD phytoplasma and SCYLV were confirmed in most of the clumps showing unspecific yellowing and stunting of sugarcane vars. CoSe 92423 and CoSe 93232.

However, no incidence of GSD and SCYLV was recorded in affected crops of other districts in the study. In these fields Pyrilla was considered as main cause of yellowing and stunting. But the stunting with GSD-SCYLV-Pyrilla complex was more severe and the growth was completely inhibited with side shooting with no shoot formation giving a bushy appearance of the affected clump. Because of no clear symptoms of GSD/SCYLV, it was very difficult to identify the diseases on symptom basis. Only through PCR assays, the existence of both these pathogens could be confirmed. Our results proved that the unspecific yellowing and stunting in Maharajganj districts of eastern Uttar Pradesh, India was caused by GSD-SCYLV-Pyrilla complex and was responsible for 100% yield losses of the affected sugarcane varieties. This needs to be properly investigated for checking the further spread of this disease-pest complex in other potent commercial varieties. During survey in last week of July and first week of August, heavy infestation of Epipyrox, the natural parasite of Pyrilla was observed in all the infested fields in all the abovementioned districts. This way the Pyrilla infestation was being recovered but we should be very attentive for those fields where unspecific stunting would exist even after the recovery of the Pyrilla. Our results suggests that Pyrilla infestation in combination with virus and phytoplasma could be more harmful and cause significant losses to sugarcane crop under favorable climatic condition for pest and disease development.

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Effect of brown rust on sugarcane yield in Louisiana
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The effect of brown rust, caused by Puccinia melanocephala H. & P. Sydow, was studied in two fields of cultivar LCP 85-384. Yield was compared in plots naturally infected or treated with three combined fungicides, azoxystrobin, propiconazole, and tebuconazole, applied every two weeks. To evaluate disease impact during different epidemic stages, treatments were initiated and stopped at different times. Six treatments were included: no fungicide application and fungicides applied in April only; during April and May; during April, May and June; during May and June; and during June only. Fungicides were applied to the center two rows of four, 18.3 m row plots with four replications. Rust severity was determined by image analysis as mean percent leaf area occupied by lesions on 10 leaves per plot. Yield data was collected from treated rows for stalk population, weight, and sucrose content; cane tonnage; and kg sucrose/ha. At location one, rust was first observed 20 April (< 1% infected leaf area). The epidemic became visually evident by 19 May (12.0% infected leaf area) and increased to 24.4 and 22.0% leaf area infection on June 1 and 19, respectively. In comparison, infected tissue means for treated plots were 3.7, 0.6, and 1.9% for the April only, April-June, and May-June treatments, respectively. On 1 and 19 June, respectively, infected tissue means for the April only, April-June, May-June, and June only treatments were 19.8, 4.9, 3.0, and 2.3% and 24.4, 1.5, 1.2, and 2.9%. At location two, rust was not observed until 21 May then increased to 15.9% infected leaf area in untreated plots by mid-June. Infected tissue means did not exceed 1% in treated plots. At location one, stalk weight, tonnage and kg sucrose/ha were lower in non-treated plots compared to plots treated April-June with fungicides. Reductions of 17.3, 17.3, and 21.7%, respectively, were recorded. Yields were not improved in plots treated with fungicides only during the beginning of the epidemic (April) or only during the end of the epidemic (June). Yields were similar in plots treated April-June and May-June. At location two, only stalk weight was lower in non-treated plots compared to plots treated May-June. The results demonstrate that brown rust can significantly reduce yield of LCP 85-384, the current number one cultivar in Louisiana. The magnitude of the loss caused by rust suggests that the potential for economic control with fungicides needs to be explored.

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Session 4: Disease management and plant resistance

SCYLV: implications for a plant breeding programme
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SCYLV was first detected in South Africa in 1997. At that time the virus was largely restricted to the northern regions, being found in some commercial varieties and certain genotypes undergoing selection in Pongola. The source of infection is now thought to be principally varieties imported from the USA and propagated at Pongola during the late 1980s. More recently the disease has spread to the south, but is still more prevalent in the northern irrigated areas. A survey of the industry revealed that more than two thirds of varieties grown in the north are infected with SCYLV, and approximately a quarter of varieties grown in the southern areas are infected. While other countries have reported significant yield loss in SCYLV infected cane, the effect of the virus on South African varieties is not yet known with certainty. SCYLV appears to spread rapidly on the Pongola Plant Breeding Selection farm from infected to uninfected susceptible plants when these are in close proximity. Transmission over greater distances takes more time and it may be several years before a particular genotype becomes exposed or infected. There is circumstantial evidence for yield loss ranging from 3 to 16% depending on genotype and season. Slow transmission and yield loss both have implications for the SASRI plant breeding programme, in that yield-based selection is implemented early in the selection programme, before selections have become exposed or fully infected. It might be beneficial to ensure that every clone has been exposed at the single stool stage, such that subsequent yield-based selection could result in varieties for release that are either immune (e.g. N25) or 100% infected and tolerant (e.g. N32). The release of intolerant varieties, which subsequently become fully infected (e.g. N30), should be avoided since growers could experience yield decline, and the 'lifespan' of such varieties would be greatly reduced. In this presentation advances in the detection of SCYLV and tissue culture for the 'curing' of infected material are also discussed.

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Studies on the Sugarcane yellow leaf virus (SCYLV) in Colombia
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The sugarcane yellow leaf virus is a pathogen of economic importance in the Cauca Valley, Colombia. The causal agent is a Polerovirus of the Luteoviridae family. During 2004, the disease incidence was of 12.2 % in commercial fields. Due to the

importance of this disease, CENICAÑA has established through the time strategies for its management: A methodology of the virus purification, adding 2% Celluclast 1.5 L to the extraction buffer followed by using a 10-40% of sucrose gradient was established. The obtained antigen was used in the production of immunoglobulins by injecting New Zeland rabbits. The produced antibody is used for DBIA, TBIA and ISEM. The transmission of the virus was studied with two species of aphids: Melanaphis sacchari and Rhopalosiphum maidis. Insects acquired the virus by 48 hours in infected plants of variety SP 71-6163 and transmission occurred in 5 days, using of 10-20 healthy plants of the variety SP 71-6163, sorghum Rio variety and sweet corn. The M. sacchari showed its efficient vector capacity of the virus by infecting 51% of sugarcane plants, 19% of sorghum and 42% of corn, with incubation periods between 45 and 90 days. The R. maidis transmitted the virus to 30% of the sugarcane plants, to 10% of sorghum and 10% of corn, with periods of incubation between 60 and 90 days. The presence of races of the SCYLV in the Cauca Valley was determined using the methodology of the RT-PCR and the specific ARN of affected varieties and primers o-FM359/323. The results showed a 1200 pb band of amplification in samples from the tested varieties. When digesting the amplified bands with the Sau 3AI restriction enzyme, bands with size of 100 pb and 200 pb were found which corresponds to the race of Brazil or a fragment of 300 pb which is characteristic of the Texas-Florida race. Samples from some varieties presented simultaneously three bands which could correspond to a new race of the virus. In order to determine the presence of the SCYLV in weeds and insects found around commercial fields of infected sugarcane varieties, samples were collected and then evaluated by the TBIA, DBIA, RT-PCR and electronic microscopy. The results did not show the presence of the virus in host plants and evaluated insects. Studies of resistance in sugarcane showed that varieties CC 84-75, CC 87-505, PR 61-632 are susceptible to the disease, whereas variety CC 85-92, main variety cultivated commercially has excelled by its resistance to the SCYLV. At the moment transformed lines of the CC 84-75 are in an isolated field and so far have not shown infection by the SCYLV.

Keywords: Yellow leaf virus (SCYLV), antiserum, insect transmission, races, host, resistance

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Ultrastructural alterations elicited by Sugarcane bacilliform virus in leaf tissues of Venezuelan sugarcane cultivars

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In Venezuela, the infection of sugarcane cultivars by the Sugarcane Bacilliform Badnavirus (SCBV) was first reported in 2001 (Garrido, Ordosgoitti and Lockhart, Fitopatología Vegetal 16: 73, 2003). This virus together with the sugarcane mosaic virus (SCMV) and the Sugarcane yellow leaf virus (Izaguirre, J. Phytopathol 150: 13-19, 2002) constitute the main constrains for crop yields in main agricultural areas. Serological analyses indicate that SCBM infects the sugarcane cultivars Salangore, D15841, V6410, C32668, B63168, CP29291, CP742005, CP721210 and CP31588. Leaf symptoms in SCBV-infected cultivars are unconspiscuos in most cultivars and plant growth does not seem to be hindered by the SCBV. Farmers, however, report a reduction in the plant sugar content. The latter observation could be ascribed to the cell ultrastructural alterations detected in SCBV-infected leaf tissues. Mesophyll chloroplast did not have the typical elongated form, were found detached from the plasma membrane, with compact grana characterized by a very short non-appressed (unstaked) lamellae. Chloroplasts were also found filled with small osmiophilic lipid globuli, but were depleted of starch grains. Concomitantly, the plasma membrane seemed detached from the cell wall, the central vacuole was irregular in shape, and there was a marked reduction in the number of mitochondria per cell which in turn were swollen with a cristae system completely distorted. In contrast, the ultrastructure of the nuclei did not seem to be hampered, with the heterochromatin concentrated near the periphery while the euchromatin was located toward the center of the nuclei. Virus inclusion bodies were not detected so far.

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Prediction of disease resistance ratings for fiji leaf gall and smut using NIR spectroscopy

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Fiji leaf gall is one of Australia's most serious diseases of sugarcane and represents a significant problem in almost half of the total area under sugarcane production. Likewise, smut is a devastating disease that has recently entered Australia, although it is not yet present in the major sugarcane growing regions of Queensland. Rating sugarcane clones for resistance/susceptibility to Fiji leaf gall and smut is difficult and expensive. We investigated NIR spectroscopy as an alternative means to predict the rating of clones without requiring specific field trials. For Fiji leaf gall, ten standard varieties were sampled on two sampling dates and the NIR spectra of the spindle leaf was measured and the data subjected to partial least squares (PLS) analysis. The spectra from one of each replicate were included in a calibration/training set with the remaining replicates used for model validation. The predicted versus measured ratings were strongly correlated with an R2 = 0.942, and the model provided acceptable predicted ratings for all clones. The standard error of prediction (SEP) was determined to be 0.90, which is more than acceptable compared to the alternative testing methods. A similar method was used to assess NIR for predicting the resistance of varieties to smut, with spectral measurements taken on the bud scales. Five different varieties with resistance ratings ranging from 1-9 were examined with similar data treatment to that outlined above. Again, promising results were observed from the PLS analysis, with a strong correlation observed and an acceptable standard error of prediction of 0.74. Further research intends to develop these preliminary results into a tool(s) for use within the plant breeding and selection program to screen at an earlier stage(s) for resistance, thereby providing cost savings, productivity benefits and increased numbers of resistant clones to later selection stages. The primary advantages of this method are the timescale to produce ratings (potentially hours as opposed to months) and that requirements of specific field trials, suitable infection levels and quarantine will be minimal. Other prospects exist to develop improved understanding of the basis of resistance

to Fiji leaf gall and to develop similar screening technologies for other pest/disease resistance issues that are either difficult or expensive to rate for using traditional techniques.

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Genetic mapping of sugarcane resistance to smut through bi-parental segregation and associations among modern cultivars

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Breeding for smut resistance is efficient because this trait is fairly heritable but it requires complicated screenings. Moreover, the genetic control of smut resistance is still unknown. With the objective to identify the mendelian factors involved in sugarcane resistance to smut, two strategies have been implemented (1) QTL mapping in a bi-parental progeny derived from a cross between a resistant cultivar 'R 570' and a highly susceptible clone 'MQ 76/53', evaluated in Reunion island for resistance to smut (2) Association study in a population of cultivars, evaluated in Burkina Faso for resistance to smut. The genetic maps of the two parents of the bi-parental progeny, 'R 570' and 'MQ 76/53', were constructed using a population of 198 progeny. A total of 1666 polymorphic markers were produced using 37 AFLP primer pairs combinations, 46 SSRs and 9 RFLP probes. Linkage analysis allowed the construction of 86 cosegregation groups for 'R 570' and 105 cosegregation groups for 'MQ 76/53' encompassing 424 and 536 single dose markers respectively. The cumulative length of 'R 570' map was 3144 cM. The cumulative length of 'MQ 76/53' map was 4329 cM. Field trials and greenhouse trials using different inoculation methods were conducted in order to characterize the resistance of the 198 progeny clones from the bi-parental population. The distribution of disease scores observed in all those trials was highly unbalanced toward the resistant parent indicating the segregation of multiple dominant resistance factors. A QTL detection was performed using the 1666 available markers allowing the identification of a few genomic zones with small effects. The structure of linkage disequilibrium in the population of 74 cultivars was investigated using 1626 AFLP markers, among which 408 have known positions on 'R 570' genetic map. This analysis confirmed that linkage disequilibrium in sugarcane extends over distances of tenth of centiMorgans but drops sharply for distances over 5 cM. This order of magnitude makes genome-wide association studies achievable in sugarcane. The association study performed in the population of 74 cultivars (constituted of two subpopulations, one highly susceptible to smut and the other highly resistant to smut) revealed interesting haplotypes associated with resistance. Two QTLs have been detected through both approaches. The progress obtained toward a better understanding of the genetic determinism of sugarcane resistance to smut is modest. The potential of association studies in sugarcane appeared interesting although much more markers and an extended population would have to be used to make the most of it.

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Session 5: Epidemiology and disease spread

Temporal increase and spatial distribution of yellow leaf and sugarcane aphid infestations

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Sugarcane yellow leaf, caused by Sugarcane yellow leaf virus (SCYLV), is a potentially important disease recently introduced into Louisiana. A state-wide survey to determine the distribution of SCYLV-infection showed that virus was present in all areas of the Louisiana sugarcane industry. Incidence averaged 15% in fields with infected plants. However, virus was not detected in 52% of the fields. Disease progress curves determined in four fields during two growing seasons indicated the greatest temporal increase of yellow leaf occurred during late spring and early summer which coincided with the initial infestation and increase of the sugarcane aphid, Melanaphis sacchari. Aphid infestations in 2002 and 2003 ranged from 1.2 to 53.7 and 1.3 to 6.6 aphids per leaf, respectively. Final disease incidences of 2.9, 5.2, and 5.2% were recorded in three fields planted with virus-free seedcane. Incidence in a fourth field increased from 12.2 to 25.9% during the two season study period. Spatial distribution of SCYLV infections and aphids were evaluated in contiguous quadrat grids with spatial autocorrelation analysis. Both aphids and SCYLV exhibited predominantly random distribution with occasional aggregation suggesting limited secondary spread occurred within fields. The low incidence and rates of disease increase observed despite the widespread occurrence of potential vectors suggest that inoculum pressure is still low in Louisiana. The results suggest it may be possible to keep yellow leaf at low levels by planting virus-free seedcane.

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Spatiotemporal evolution of plant infection by SCYLV in a disease free plot. Toward modeling virus spread in tropical conditions

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A sugarcane trial (17 rows of 55m each) was established with 1,745 disease-free tissue culture propagated plants of cultivar SP71-

6163. In Brazil, this cultivar is highly susceptible to yellow leaf caused by the Sugarcane yellow leaf virus (SCYLV). Two rows of SCYLV-infected plants of cultivar FR90714 were also planted next to one side of the SP71-6163 plot. The number of SCYLV-infected plants was monitored on weeks 6, 10 14 19 and 23 after transferring plants to the field, on all plants by tissue blot immunoassay (TBIA). Colonization of disease-free plants by aphids was monitored in plant cane crop and aphid population structure was estimated from 40 random identified plants. Alate aphids were observed 2 days after transferring plants in the field and increased during 8 weeks with a mean of 2 alate aphids per plant and then decrease to a mean of 0 to 0.3 alate aphids per plant on week 15 until end of observation on week 23. The first SCYLV-infected leaves were found on 6 plants, 6 weeks after planting. Number of infected plant then increase slowly up to 4% of the plants until week 14 with no aggregative contamination but distribution of infected plant within the field was heterogeneous. Then infected plants drop to 12.6% on week 19 and end at 18% of the plants on week 23. The two last samples showed short distance aggregation between infected plants with maximum signification within a specified distance of 2m between plants. Random infection was linked to high level of alate aphid population and was followed by neighbourhood infection due probably to movement of aphids from plant to plant when apterous aphid population increased and was observed on all plants. Results showed that primary infection of disease free plot due to alate aphids is low and happens during the first stage of growth (3 to 4 months). Main infection is due to disease expansion from primary infected plants.

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Transmission of Sugarcane yellow leaf virus and Sugarcane mosaic virus in Ecuador

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The two major virus diseases of sugarcane in Ecuador are yellow leaf caused by Sugarcane yellow leaf virus (SCYLV) and mosaic caused by Sugarcane mosaic virus (SCMV). Incidence of yellow leaf in commercial fields is high and the disease is widespread in the country. Mosaic is found in fewer locations but, when present, yield losses can reach 1.14 tons of cane/ha per 1% incidence. Experiments were conducted to identify insect vectors of SCYLV and SCMV in Ecuador, and to determine optimal acquisition times of viruses by insects and vector preference. Seven ECSP selected sugarcane clones and four commercial varieties were used to determine the insect vector variety preference. The virus transmission was verified by tissue blot immunoassay (TBIA) and dot-blot immunoassay (DBIA). Additionally, screening for mosaic resistance was performed by mechanical inoculation with the CINCAE sugarcane variety collection. SCYLV was transmitted by the white sugarcane aphid, Melanaphis sacchari Zehnt, but not by the sugarcane plant hoper Perkinsiella saccharicida Kirkaldy. SCMV was transmitted by the corn leaf aphid, Rhopalosiphum maidis Fitsh, but not by the yellow sugarcane aphid, Sipha flava Forbis, or P. saccharicida. Efficient transmission of SCYLV by M. sacchari was obtained with 0.5 hr for insect fasting, 48 hr for virus ingestion access period from the host plant and 0.5 hr for the inoculation access period. Efficient transmission of SCMV by R. maidis was obtained with 2 hr for virus ingestion from the host plant, 0.5 hr for insect fasting period and 0.5 hr for the inoculation access period. ECSP98-169, ECSP98-127 and PR 67-1070 are the sugarcane clones that were preferred by M. sacchari, with 108, 96 and 101 aphids per plant, respectively. These results suggested that healthy plants of these clones will be rapidly infected by SCYLV in the field. On the other hand, clone ECSP98-392 was not much colonized by R. maidis, with only 10 aphids per plant. Sixty days after mechanical inoculation, 60% of sugarcane varieties from the CINCAE collection were ranked resistant to SCMV. Promising parental clones are therefore available for the breeding program of CINCAE.

Keywords: Transmission, Potyvirus, Polerovirus, Saccharum sp., DBIA, TBIA.

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Studies on the acquisition of Fiji disease virus byY Perkinsiella saccharicida

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Fiji disease virus (FDV) is transmitted by planthoppers in the genus Perkinsiella and causes Fiji leaf gall (FLG) disease of sugarcane. FLG can cause total crop loss in susceptible varieties and epidemics in Australia and Fiji have threatened the viability of the sugar industries in these countries. In Australia, the only species of planthopper known to transmit FDV is P. saccharicida. In this study we tried to understand the factors that influence the acquisition of FDV by P. saccharicida. Virus acquisition is an important part of the epidemiology of the disease because it influences the proportion of the vector population that can potentially spread the disease. Our study has shown that:

- Virus acquisition can vary between populations of P. saccharicida. Populations from North Queensland that have never been exposed to the virus had significantly higher rates of acquisition compared to populations from South Queensland.
- The FLG-infected cultivar used as the source for acquisition of the virus by the planthoppers significantly influences the proportion of planthoppers that acquire the virus. There is a strong relationship between cultivar resistance to the disease and suitability as a source of acquisition of the virus by the planthopper. Susceptible cultivars are superior as source plants for acquisition.
- The virus titre in different cultivars is not related to acquisition. Virus titre was measured by quantitative PCR in gall and non-gall tissues and there was no significant difference between cultivars. Galls contained 200 times more virus than non-gall tissue.
- Acquisition was related to the proportion of leaf area covered by galls. The severely stunted leaves of susceptible varieties had a much higher proportion of their leaf area covered by galls. This suggests that the probability of a planthopper feeding on a gall would be higher in these plants.

- The severity of disease symptoms in infected plants was correlated with resistance.

Our study has shown that susceptible varieties not only increase the chance of an epidemic by having a greater probability of becoming infected but infected plants of susceptible varieties will breed more infective planthoppers. It appears that acquisition of the virus is related to feeding behaviour of the insect and not to virus titre in the plant. Populations of the planthopper vary in their ability to acquire the virus with a population from North Queensland that has never been exposed to the virus being the best at acquiring the virus. This suggests that if FDV arrived in North Queensland the native population of planthoppers would spread the disease successfully.

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Impact of rainfall on epiphytic colonization of sugarcane by the leaf scald pathogen and associated plant infection

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Colonization of the sugarcane leaf canopy by Xanthomonas albilineans appears to be an important step in the epidemiological cycle of leaf scald disease in Guadeloupe. Previous studies showed that healthy sugarcane plants can be infected by X. albilineans after aerial transmission of the pathogen. Impact of climatic conditions on variation of colonization of the sugarcane leaf canopy and subsequent stalk infection by the pathogen is, however, unknown. Trials were set up in Guadeloupe in 3 different locations with cultivar B69566, susceptible to leaf scald, but still grown commercially in Guadeloupe. Disease-free tissue-culture propagated sugarcane plants were transferred to the field in 1999. Epiphytic populations of X. albilineans were regularly monitored for 3 crops (plant cane and two ratoons) by measuring bacterial populations in water droplets sampled from the sugarcane leaf surface. Infection of sugarcane stalks by X. albilineans was determined by isolating the pathogen from the stalk sap after 11-12 months of growth in each crop cycle. In plant cane, the first detection of the pathogen on the leaf canopy varied according to proximity of other sugarcane fields and climatic conditions. Additionally, once the leaf canopy was entirely colonized, epiphytic population sizes of the pathogen also varied between crop cycles and locations. Maximum population sizes were observed at the end of the wet season (November-December). These populations ranged from 2 to 107 bacteria per ml of water droplet according to crop location and crop cycle. Bacterial populations on the leaf surface were correlated with total rainfall during the wet season (first half of the crop cycle) and percentage of stalk infection by X. albilineans varied in accordance with epiphytic populations of the pathogen. The amount of rainfall therefore appeared critical for the epiphytic phase of sugarcane leaf scald and subsequent stalk infection. If these results can be confirmed with other sugarcane cultivars, amount of rainfall could be used to predict stalk infection after aerial transmission of the leaf scald pathogen.

Top

Poster Session

Ultrastructural studies of a sugarcane somaclone resistant to Sugarcane Mosaic virus in Venezuela

Maira Oropeza and Eva de García

Sugarcane is the most important source of sugar in Venezuela. All commercially - high yield cultivars recommended to farmers are, however, susceptible to infection by the world wide aphid-spread Sugarcane Mosaic Virus (SCMV), strains A and B. In Venezuela this virus is endemic to sugarcane crops since 1940 causing severe economical losses to farmers. This investigation was, therefore, carried out to obtain local resistant cultivars. As a result, a sugarcane somaclone AT626 resistant to SCMV was obtained from the cultivar PR62258 using in vitro culture techniques. The resistance to infection proved to be stable over nine years of green house and field trials in which plants were asexually reproduced and mechanically inoculated with the SCMV. Further studies revealed that the resistance to SCMV infections in the somaclone AT626 seems to be related to the presence of thicker cell walls which have an average of 937 nm in comparison to the 783 nm observed in the cultivar PR62258. Concomitantly, secondary cell walls in the somaclone AT626 were highly suberised and lignified. Whereas, in the cultivar PR62258 cell walls only showed a suberin lamella. These anatomical observations indicate that genetic breeding for thicker cells walls might be an effective mean to control the infection of the sugarcane plants maybe not only by SCMV but also by other aphid-transmitted viruses capable to infect this important crop.

Top

Transformation of Ustilago scitaminea with a GFP gene

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Sugarcane smut disease, caused by the fungus Ustilago scitaminea is time consuming and costly to control. Breeding resistant varieties is ongoing and would be made more cost effective if susceptible varieties could be pinpointed and eliminated much sooner than current methods permit. The aim of this project is to develop a new diagnostic test using a fungus transformed with a GFP gene. The GFP gene will allow researchers to observe the fungus in the plant with UV light at an earlier stage of infection. U. scitaminea haploid sporidia were transformed by first treating them with lytic enzymes to produce spheroplasts. The plasmid pOTEF-SG [Spellig, 1996 #1205] containing the SGFP-TYG gene under the control of the synthetic OTEF promoter was introduced by polyethylene glycol mediated transfection. Selection was carried out on media containing hygromycin B. Hygromycin resistant colonies were tested for the presence of the GFP and hpt genes and examined by epifluorescence microscopy. Transgenic colonies were identified which produced a strong green fluorescence when excited by UV light at 470nm. These transformed haploid cells will be mated with opposite mating type haploid cells to form infectious transformed dikaryon for plant infection experiments. If the GFP-transformed U. scitaminea enables monitoring of the course of infection in sugarcane varieties with different levels of resistance, this method could permit the rapid evaluation of new varieties for smut resistance.