

pear trees, and *L. crescens* BT-1 (Lcr, isolated from mountain papaya). Unlike the other Liberibacters, BT-1 has been cultured and sequenced (GenBank CP003789) and thus has the potential to be developed into a model system. To determine if BT-1 might be tractable for functional genomics studies, the minimum inhibitory concentrations (MICs) of several antibiotics commonly used for plasmid selection was determined. BT-1 was found to be quite sensitive to: chloramphenicol, < 4 mg/L; gentamycin, < 1 mg/L; kanamycin, < 2.5 mg/L, and tetracycline, < 0.3 mg/L. Both the repW (on pUFR071) and *Bordatella* replicons (pUFJ05, derived from pBBR1MCS5) were transformed by electroporation at high frequencies into BT-1. Stability of pUFR071 was evaluated; this plasmid was >95% stable, without selection, when grown in BM7 medium for over 20 generations. pUFR071 was extracted from BT-1, retransformed into *E. coli* and appeared from restriction analysis to be unchanged. Attempts to artificially inoculate marked strains into tobacco, citrus and periwinkle are currently in progress.

Pan-genome analysis of *Xanthomonas citri* subsp. *citri* provides insights into bacterial evolution and pathogenicity

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Phytopathology 103(Suppl. 2):S2.67

Citrus canker is a devastating disease that affects most commercial citrus varieties. Asiatic (A) type citrus canker is the most widespread and destructive form of citrus canker and is caused by *Xanthomonas citri* subsp. *citri*. Multiple variants of XccA including XccA^w and XccA* were reported. Compared with XccA, XccA^w and XccA* are limited in host range. To investigate the genomic variation and evolution of *X. citri* subsp. *citri*, a pan-genomic analysis was performed on 21 newly sequenced *X. citri* strains and three other reference strains representing distinct geographic, temporal, and host of origin. Analysis of these genomes showed that the pan-genome of *Xanthomonas citri* subsp. *citri* strains consists of a core genome shared by all isolates, accounting for about 85% of a single genome. The chromosomes of all the XccA strains were highly homogeneous. Comparatively, greater genetic diversity was observed in XccA^w and XccA* strains, with the majority being attributed to deletion and transposition events associated with insertion sequences and plasmids. We will present our current progress in understanding the evolution and pathogenesis of XccA based on comparative genomic analyses.

The development of a phage therapy for the control of the causal agent of horse chestnut bleeding canker, *Pseudomonas syringae* pv. *aesculi*

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Phytopathology 103(Suppl. 2):S2.67

Bleeding canker of horse chestnut trees poses a great threat to the species in the U.K. and the rest of Europe. A large number of trees in the South East of England have the disease and as yet no control strategy has been found. Without appropriate intervention we may face the loss of an amenity species. To identify novel control strategies requires considerations of new therapies, especially since antibiotic usage is restricted. Phage therapy offers the opportunity for a natural, chemical-free biocontrol agent. A number of bacteriophages from diseased and non-diseased tree material from the South East of England were isolated. These have the ability to infect and lyse a number of isolates of the causative agent of this disease, *Pseudomonas syringae* pv. *aesculi* from the U.K. and Europe and some other *syringae* pathovars. A subset of 4 of these phage were further characterised and discovered to be tentatively belonging to the order Caudovirales and the families Myoviridae and Podoviridae, based on TEM and genetic sequencing results. These phage isolates were evolved in co-evolution experiments with the host to create new genotypes to combat bacterial resistance to the therapy. This will hopefully mean the development of a viable phage therapy strategy for the control of bacterial bleeding canker.

Wound response and ROS production in blue mold resistant wild apple germplasm from Kazakhstan and central Asia

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Phytopathology 103(Suppl. 2):S2.67

Blue mold caused by *Penicillium expansum* is the most destructive pathogen of stored apples in the U.S. and worldwide. It was recently shown that resistance to blue mold exists in wild apples, *Malus sieversii*, from Kazakhstan and central Asia maintained in a germplasm collection in Geneva, NY. We initiated studies to determine the mechanism of resistance to *P. expansum* in select wild apple accessions. Wound responses (up to 96 h in 24 h intervals), affecting *P. expansum* infection, and related cytological changes were observed in accessions with varying resistance levels. In general, the more resistant the accession, the quicker the wound response that prevented fungus from infecting tissue and causing decay. On moderately resistant accessions, a 24 h interval between wounding and inoculation was sufficient to avert decay. More time was needed for the wounds to develop resistance to higher inoculum concentrations of the pathogen. No decay developed on immune apple accessions, even when inoculated immediately after wounding. Reactive oxygen species (ROS) were detected at high level immediately after wounding in the immune accessions, and gradually accumulated around the wound over time in both resistant and moderately resistant accessions. Results from this study suggest an involvement of ROS in the mechanism of resistance to *P. expansum* in select wild apple accessions.

Pandemic, novel, and hybrid genotypes of the amphibian pathogen *Batrachochytrium dendrobatidis* in the southern Atlantic Rainforest of Brazil

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Phytopathology 103(Suppl. 2):S2.67

Chytridiomycosis of amphibians, caused by the chytrid pathogen *Batrachochytrium dendrobatidis* (*Bd*), is the emerging infectious disease implicated in the recent population declines and extinction of amphibian species worldwide. Current data suggest that *Bd* has been recently introduced to naïve host populations by the intercontinental movement of amphibians. In the decade after its discovery in 1998, all isolated strains of *Bd* were shown to belong to a single, hyper-virulent clonal genotype. Recently however, multiple groups have reported the existence of novel *Bd* genotypes, including the existence of a hybrid genotype from the Atlantic Rainforest of Southeastern Brazil. In-depth sampling is needed to define the spatial distribution of the putative endemic and hybrid genotypes. To investigate the population structure of novel, pandemic and hybrid lineages in this putative hybrid zone, pure isolates of pathogen strains were collected from *Bd* infected anurans at six field sites along a 1400 Km transect of the historical range of the Atlantic Rainforest. Isolates of *Bd* strains were genotyped by multi-locus sequence typing to assess the effect of latitude, presence of invasive amphibian species, and anthropogenic habitat fragmentation on the spatial distribution of *Bd* genotypic diversity. Insights gained from this investigation will provide a future framework to test the hypothesis that the hyper-virulence of the pandemic lineage arose from past hybridization events.

CRISPR systems in plant pathogens: A new tool for epidemiological surveillance

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Phytopathology 103(Suppl. 2):S2.67

Rapidly evolving genetic loci, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), are of special interest to develop new molecular typing tools. The widely distributed CRISPR loci consist of highly conserved DNA repeats that are interspersed by unique, similarly sized spacers which originate from previous attacks by viruses and/or plasmids. For efficient management of plant diseases, knowledge about the pathogen's population structure and tools for epidemiological surveillance are prerequisite. We wish to exploit CRISPR loci as a molecular typing tool of plant

pathogens, as exemplified by *Xanthomonas*, an important clade of Gram-negative bacteria infecting a plethora of plants, including rice, cereals, cassava, banana and citrus fruits. CRISPR loci of 56 *X. citri* subsp. *citri* strains of world-wide origin were sequenced, revealing a repertoire of 37 unique spacers. A dendrogram based on the presence and absence of spacers was largely congruent with previous typing using AFLP. Our results demonstrate that CRISPR-based spoligotyping can be used as an efficient and robust method to study the phylogenetic relationships among isolates of plant-pathogenic xanthomonads, such as the citrus cancer pathogen *X. citri* subsp. *citri*. Implementation of *Xanthomonas* spoligotyping will serve phytosanitary measures by assisting the epidemiological surveillance of outbreaks of citrus canker and other diseases.

Diversity of Blastocladiomycota and Chytridiomycota of the “Parque Estadual da Ilha do Cardoso” (PEIC), Cananéia, São Paulo State, Brazil
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Phytopathology 103(Suppl. 2):S2.68

Here, we report the results of a study to analyze and compare the richness and diversity of Blastocladiomycota and Chytridiomycota in freshwater and soil ecosystems of the “Parque Estadual da Ilha do Cardoso” (PEIC), which is a protected fragment of the Atlantic Rainforest area located in Cananéia municipality, São Paulo State. On two sampling dates (winter and spring), we collected water and soil samples at 15 different points. These samples were processed in the laboratory by using cellulosic, chitinous and keratinous substrates. Of the 60 samples analyzed, we obtained 155 isolates of 31 morphological species, 29 taxa belong to the Chytridiomycota and two to the Blastocladiomycota, 26 of them were identified to the species level. Approximately 30% of the isolates were purified in culture media and characterized on the basis of morphological and molecular data (18S, ITS and 28S regions of the rDNA). We performed the first phylogenetic analysis of *Nowakowskiella elongata*, *N. multisporea* and *Cladochytrium tenue*. *Chytrium lucidus* was noted for the first time in Brazil and 94% of identified species are first records for the PEIC. Our results demonstrate the importance of biodiversity inventories, particularly in South America, where the knowledge of zoospore fungi is scarce. Financial support: CAPES (grant)/FAPESP.

Diversity of zoospore assemblages from Pirarungaua stream, São Paulo, Brazil

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Phytopathology 103(Suppl. 2):S2.68

The present study aimed at surveying the zoospore assemblages (Fungi and Chromista) of the Pirarungaua stream, “Parque Estadual das Fontes do Ipiranga” (PEFI), which is one of the few remnants of Atlantic Forest in urban areas of São Paulo city. At six stream sites, water and mixed leaf litter were collected monthly from June 2011 to April 2012. Samples were processed in the laboratory by a multiple baiting technique using cellulosic, keratinic and chitinous baits. A total of 676 isolates was recorded, 309 from water and 367 from leaves. The isolated taxa were morphologically identified and incorporated either into the Fungal Culture Collection or the Herbarium of the “Instituto de Botânica”. Forty-two taxa were recovered, 27 identified to the species level (15 Chytridiomycota, one Blastocladiomycota and 11 Oomycota). Of the total taxa, seven are new records for the PEFI and one for Brazil. Richness, evenness, Shannon’s and Simpson’s indices were high from both water and in the mixed leaf litter, with a high similarity based on Sorensen’s index. We thank National Council for Scientific and Technological Development (CNPq) for financial support.

Effect of quinolone alkaloids isolated from *Esenbeckia alata* and *Raputia heptaphylla* (Rutaceae) on *Botrytis cinerea*

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Phytopathology 103(Suppl. 2):S2.68

Botrytis cinerea is the fungus responsible for the gray mold disease, which affects an important amount of crops worldwide. In Colombia, gray mold causes 76% of the losses of fruit and ornamental crops. In previous studies, quinolone alkaloids from plants of the family Rutaceae showed biological activity against several plant and human pathogens. In this work, 9 quinolone alkaloids, isolated from *R. heptaphylla* and *E. alata*, were evaluated *in vitro*

against *B. cinerea*. Evaluation included a test was aimed to determine the effect of alkaloids on mycelial growth, and a second test to determine the effect on conidial germination. Five out of nine substances tested were found to be active against the pathogen by inhibiting its growth and reproduction. Conidial germination and mycelial growth were inhibited almost in a 100%, in few cases conidial germination was observed but sclerotia, instead of aerial mycelium, was formed. Quinolone alkaloids that showed activity against *B. cinerea* were used in an additional *in silico* study, aimed to elucidate their putative target. Active sites of enzymes, reported as target for some fungicides and acquired from PDB, were chosen for molecular docking simulation. Five alkaloids exhibited better affinity scores for cystationine γ -lyase and cytochrome-b. This preliminary result will be used as a working hypothesis in the following experiments in order to delineate the mechanisms of action for quinolones alkaloids on *B. cinerea*.

Genetic differentiation between *Verticillium dahliae* populations from asymptomatic and symptomatic hosts

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Phytopathology 103(Suppl. 2):S2.68

Verticillium dahliae (Vd) causes disease on over 400 hosts. Monocotyledonous crops have been traditionally considered to be non-hosts of Vd, and cereal species have been used in rotations as a strategy to manage *Verticillium* wilts. When sampling oats planted in with potatoes in PA fields with a history of *Verticillium* wilt, we found Vd growing endophytically; the fungus was isolated from surface-disinfested internal stem tissue of asymptomatic oat plants. A population genetics approach was used to examine Vd obtained from potato and oats grown in rotation in the same fields. Microsatellite marker analysis showed that the populations were significantly different. While at least seven distinct Vd genotypes were present in the populations from potato samples, only one of those genotypes was consistently found associated with oats, in different sampling years and from different fields. These results indicate that the interaction between Vd and oats is highly specialized; not every Vd genotype seems to be able to establish an endophytic relationship with oats and possibly other monocots, but this is potentially restricted to certain Vd genotypes. The dual role of Vd, pathogenic on certain plants and endophytic on others, raises interesting questions about the biology, ecology, persistence, and spread of this fungus, and has important potential implications in the management of *Verticillium* wilts in agroecosystems.

Possible infection of above-ground plant tissue by airborne conidia of nonpathogenic *Fusarium oxysporum*

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Phytopathology 103(Suppl. 2):S2.68

Fusarium oxysporum (Fo) is a common soilborne fungus that causes *Fusarium* wilt in plants by infection through plant roots and colonization of the vascular system. Fo can also infect plants asymptotically as an endophyte. The extent of endophytic colonization by different nonpathogenic Fo originally isolated from diverse hosts was tested by inoculating chickpea plants and recovering the fungus from surface-disinfested roots, crown, stem, and seeds of the plant. Inoculations were performed by planting pre-germinated seeds in soil infested with a given Fo genotype. Plants were grown under controlled conditions until seed set. Fo was always recovered from roots and crowns and frequently recovered from stems and seeds. However, recovered isolates were frequently different from the genotype used to inoculate the soil initially. This mismatch increased with the height of the plant, with 31% of the isolates from the crown, 63% of isolates from the stem, and 83% of the isolates from the seeds differing from the soil-inoculated genotype. These results suggest that conidia of Fo were dispersed aerially and infected the above-ground portions of the plant independently of infection that took place through the root system, possibly through infection of the flower or other above ground tissues. Although these observations were made in a growth chamber under controlled conditions, this aspect of Fo biology may have important implications in the ecology of this important fungus.

Formulation development of nontoxicogenic biocontrol strain of *Aspergillus flavus*: Wetting agent selection and physical property comparison

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Phytopathology 103(Suppl. 2):S2.68

To enhance the efficacy of *Aspergillus flavus* Afla-Guard (NRRL 21882) as a biological control agent for control of aflatoxin contamination in corn, a series of laboratory evaluations were conducted to improve/optimize Water Dispersible