

false negatives. We have optimized simple and rapid DNA extraction methods from seed wash samples for sensitive detection of tomato canker, bacterial spot and black rot pathogens. Bacterial cells are pelleted by differential centrifugation and DNA extracted using the PowerFood Microbial DNA kit from MoBio with modifications, followed by a realtime PCR. Pathogen detection sensitivities were evaluated using both naturally infected and pathogen-spiked seed samples. This DNA extraction method when combined with realtime PCR is capable of consistently detecting *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) spiked at 200 CFU/10,000 seeds. The method was validated using seed lots with different countries of origin, sanitation treatments and varying levels of saprophytic background. The newly optimized DNA extraction method in combination with realtime PCR improved pathogen detection sensitivity, specificity and reduced the assay lead time when compared to DNA extracted by standard phenol-chloroform and culture based methods currently used in seed testing.

#### **Peanut mini core collection at ICRISAT: A reality in identifying multiple disease resistance sources**

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Susceptibility to several biotic constraints, including fungal and viral diseases, is one of the major reasons for low productivity in peanut. This situation is common in several developing countries in Asia and Africa, where resource poor farmers cannot afford expensive chemical pesticides for disease management. With an objective to identify sources of multiple disease resistance in peanut, ICRISAT's peanut mini core collection (10% of core or 1% of entire collection) consisting 184 accessions was evaluated independently for two important fungal foliar diseases such as late leaf spot (LLS) and rust under field conditions during 2012 rainy season. The same set was evaluated for peanut bud necrosis disease (PBND) under late planting in the field during 2012 rainy season. Results indicated that 8 accessions showed less than 1% PBND incidence, and two accessions (ICG 2019, ICG 13858) were completely immune, compared to the average disease incidence of 25% in the trial and 40% in the susceptible control. In the rust screening trial, 3 accessions (ICG 6022, ICG 11088 and ICG 11426) were highly resistant (<3.0 rating on a 1-9 disease rating scale) and coupled with superior yields of more than 3.0 t/ha. In the LLS screening trial, ICG 11426 was highly resistant with a rating of 3.0 on a 1-9 disease rating scale, with a yield of 3.8 t/ha. These accessions can be used further in peanut breeding programs for developing multiple disease resistant high yielding cultivars.

#### **Fungal and oomycete pathogen detection in the rhizosphere of organic tomatoes grown in cover crop treated soil**

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Soil management practices, including cover crop application, affect soil and plant health through various mechanisms. Impacts on microbial communities are known to be important, but are not well understood. This field study examined the impacts of a single-season application of cover crops on pathogen populations in the tomato crop rhizosphere. The hypothesis tested was that cover crops could rapidly enhance microbial communities suppressive to pathogen growth. The study took place in MD, NY and OH in the summers of 2010 and 2011, with a total of 260 plots tested using both macroarray and T-RFLP analyses. The macroarray used in this study specifically was designed to detect over 30 pathogens of solanaceous crops, but had not previously been used for such a field study. Macroarray was able to detect certain pathogens with much greater sensitivity than T-RFLP. Results suggest that a single-season cover crop application does not significantly impact pathogen populations in the crop rhizosphere.

#### **Assessment of citrus Huanglongbing (HLB) in Dominica**

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In a citrus Huanglongbing (HLB, citrus greening) mission sponsored by FAVACA-International Volunteer Corp and facilitated by the Caribbean Agricultural Research and Development Institute and Dominican Plant

Protection and Quarantine Unit, HLB outbreak sites were visited, disease incidence and severity evaluated, and psyllid population and distribution investigated. Inspection results indicate that HLB might have been introduced by humans through the illegal movements of HLB-infected citrus to the island and had been in Dominica for some time before Asian citrus psyllid (ACP), was detected in 2007. The disease was further spread and distributed to other residential areas via propagation of infected Mexican limes and both HLB and ACP were not detected in the commercial groves during this mission. Due to a unique island climate, geographic conditions and locations of commercial orchards, and limited spread of HLB, Dominica may still have a golden opportunity to eliminate this devastating disease from the island. Eradication efforts require an outreach for the cooperation and support from the public, a nationwide survey for any HLB on dooryard Mexican limes, removal of all infected citrus from residential areas, removal of all ACP alternate host plants, certification of the state-owned citrus nursery through advanced diagnostic methods, utilization of certified citrus stocks only and release of ACP parasitoids frequently to reduce the vector population.

#### **Distinct SNPs present in the ITS2 region of *Elsinoë australis* organism detected from citrus in Florida**

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After the USDA confirmed the detection of *Elsinoë australis*, causal agent of sweet orange scab (SOS), in Texas in July 2010, similar SOS symptoms were found on fruit of many citrus species in Florida except that symptoms were always associated with injuries such as wind scars, thorn punctures/scratches and bird-pecking wounds. An *Elsinoë* fungus was isolated and inoculated onto lemon fruit with abrading the fruit surface. Although a SOS-like syndrome developed in four months, lesions did not appear on unwounded tissues nor expand beyond the wounded areas. DNA extracted from 95 symptomatic fruit was amplified and 32 produced an expected 0.4 kb DNA band for *E. australis*-specific primers. The ITS2 region (166 bp) from 10 PCR products was sequenced and found to display distinct single nucleotide polymorphisms (SNPs). Only one SNP was found to differentiate the SOS pathotype ('T') of South America and Australia from the Natsudaikai pathotype of South Korea ('C') (base position 135). All Florida specimens displayed a distinct 'C' at this base position. Additional SNPs were detected from Florida at three other base positions; 4 display 'A' for 'C' (position 15), 3 others display 'T' for 'C' (position 42), and 3 others display a deletion for 'C' (position 46). One produced a 100% match to Natsudaikai pathotype. A sequence identical to the SOS has not yet been detected in Florida, suggesting Florida *Elsinoë* isolates are not the typical *E. australis* that causes SOS.

#### ***Pseudomonas* sp. found on *Loropetalum* stem canker in Florida**

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A *Pseudomonas* sp. was isolated on over 40 retail nursery samples of *Loropetalum chinense* with rough stem cankers. The recovered strains fell into the *Pseudomonas syringae* group or *P. savastanoi* and *P. viridiflava* by LOPAT testing. Inoculation showed a hypersensitive reaction on tomato and pepper and reproduced identical canker symptoms on loropetalum stems, but not on oleander. Wound-aided inoculation caused affected stem tissue to swell and eventually rupture to form callus tissue around cankers, producing a symptom different from the galls induced by *P. savastanoi* pathovars. Both 16S rRNA and rpoD gene sequence profiles of this *Pseudomonas* sp. rendered a 99% similarity match with many *Pseudomonas* species including *P. syringae* pv. *eriotryae*, *P. amygdali*, *P. savastanoi* while the nuclear *iaa-L* gene sequence of the *Pseudomonas* sp. had only 96% match to other *P. savastanoi* pathovars. A sensitive qPCR assay that was designed to detect the plasmid-borne *iaaL* gene carried by all gall-producing *P. savastanoi* pathovars was negative, suggesting that this *Pseudomonas* sp. does not have a plasmid copy of the *iaa-L* gene. Although the initial observation of stem symptoms might have suggested *P. savastanoi* as the causal bacterium, further investigation on canker formation and gene sequence confirmed otherwise. Information on host range determination and multilocus sequence typing will aid in naming this new loropetalum stem canker bacterial pathogen.