that the assemblage of HsvB and PseB incited galls on beet, whereas the assemblage of HsvG and PthG caused galls on gypsophila in all the tested non-pathogenic or pathogenic bacteria. Thus, different combinations of two T3Es were sufficient to elicit galls on either beet or gypsophila in a host-specific manner.

Effect of seedborne *Alternaria infectoria* on susceptibility of wheat seedlings to *Fusarium pseudograminearum*
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Seedborne fungi can affect the susceptibility of seedlings to soilborne pathogens. *Alternaria infectoria* is the dominant seedborne fungus on many commercial wheat cultivars in South Africa. This study investigated the effect of seedborne *A. infectoria* on the susceptibility of wheat seedlings to *Fusarium pseudograminearum*, an important soilborne pathogen of wheat in South Africa. Seedborne *A. infectoria* was eliminated from seed of 12 wheat cultivars using hot water treatment at 45°C for 3 hrs. Four isolates each of the two fungi were used to prepare spore suspensions of 2.5×10^5 spores/ml for each fungus, as well as a spore suspension containing a mixture of the two fungi also containing 2.5×10^5 spores of each fungus/ml. Hot water and non-hot water treated seed of the 12 wheat cultivars were plated on potato-dextrose agar for 5 days to germinate. The seedlings were then dipped in either the *Alternaria*, *Fusarium* or the mixed spore suspensions and planted in a pasteurized (83°C for 60 min) sand, perlite and soil (1:1:1 mixture) growth medium in a glasshouse (25°C day, 15°C night temperatures). Control seedlings were dipped in sterile water only. Crown rot severity was evaluated 28 days after planting. Results showed that *A. infectoria* did not affect the susceptibility of wheat seedlings to *F. pseudograminearum* and will therefore not interfere with screening of seedlings for resistance/tolerance to *F. pseudograminearum*.

Establishment a gene silencing system in *Verticillium dahliae* and identification of a novel gene required for microsclerotia formation and virulence
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The genome of *Verticillium dahliae* has been sequenced and annotated, but incorrect gene annotations and unidentified transcribed regions persist. In this study, we established a dsRNA-induced gene silencing system with a plasmid carrying two convergent opposing RNA polymerase II promoters in *V. dahliae*. Subsequently, using RNA-Seq combined with reverse-transcription PCR, we identified a novel transcribed gene, Nag1, located in a cluster of putative secondary metabolic genes whose roles remain unknown. Functional analysis of Nag1 by dsRNA-mediated gene silencing revealed that loss of Nag1 significantly decreased fungal growth and conidial production. In addition, Nag1-silenced mutants exhibited obvious defects in microsclerotia formation and fungal virulence. Consistent with phenotypic observation of the reduction in microsclerotia formation, melanin production and expression of genes involved in melanin biosynthesis were markedly reduced in Nag1-silenced mutants. Overall, our data suggest that Nag1 acts as an important regulator of fungal development, pathogenicity, microsclerotia formation, and secondary metabolism in *V. dahliae*.

Pathogenicity and phylogeny of *Fusarium oxysporum* causing cucurbit wilting in Taiwan
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Cucurbitaceae is one of important vegetable and widely distributed over the word. Several important diseases are common during growth season, such as Fusarium wilt, downy meildew and anthracnoe. Among these diseases, the Fusarium wilt caused by *F. oxysproum* (*Fo*) is most important disease in cucubit production area. Currently, more than 150 formae speciales have been recoded throughout the world. Although formae speciales show the pathogenic specificity on their host, the cross-pathogenicity has been found in different formae speciales. Previous studies domonstrated that certain formae speciales of *F. oxysporum* causing cucurbit wilt could show cross-pathogencity between different cucubit. In this study, the *F. oxysproum* obtained from cucumber, bitter gourd and loofah were used to examine the cross-pathogenicty between the three cucurbits and carried out their phylogeny. The results indicated that *F. oxysproum* isolates from cucubmer and bitter gourd could infect loofah and *F. oxysporum* isolates from loofah could infect cucumber at 20°C based on root dipping method. In addition, some of loofah plants could be infected by *F. oxysporum* isolates from bitter gourd at 28°C with root dipping method. For phylogeny analysis, the *F. oxysporum* isolates from three cucurbits did not associate with host specificity based on *IGS* and *EF-1α* sequences. However, the nucleotides of secreted in the xylem (*SIX*) protein 6 could separe the *F. oxysporum* isolates from three cucurbits.

Genomic basis for host adaptation in *Puccinia striiformis*
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Stripe rust fungi, *Puccinia striiformis* f. sp. *tritici* (*Pst*) and *P. striiformis* f. sp. *hordei* (*Psh*), are devastating pathogens of wheat and barley, respectively. However, the genomic basis of their host adaptation is not clear. To understand the evolutionary mechanisms of the different formae speciales, genomic DNA and cDNA of a *Pst* isolate and a *Psh* isolate were sequenced using next generation sequencing technologies. The assembled genomes with high continuity enabled us to determine their genome differences and understand the evolutionary history of *Pst* and *Psh*. The divergence of *Pst* and *Psh*, occurred 8.12 million years ago, has been driven by high nucleotide mutation rates. Extensive losses of gene families in both *Pst* and *Psh* have occurred separately after the divergence from their most recent common ancestor, in contrast to very few gene family gains, resulting in a large number of form-specific genes. These form-specific genes have unique genomic features compared to the conserved genes, including 1) significantly shorter in length; 2) significantly less expressed; 3) significantly close to transposable elements; and 4) redundant in pathways. Moreover, 116 and 119 genes were found to be exclusively expressed in *Pst* and *Psh*, respectively. Our data indicate that the different events of gene family losses, resulting in form-specific genes, have separate *Pst* from *Psh* and that the form-specific genes are responsible for their adaptation to different cereal crops.

Functional analysis of the *MSP18* root-knot nematode virulence gene in rice
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Several root-knot nematodes (RKN) species are responsible for rice (*Oryza sativa*) production losses in Brazil, Asia or Africa. Successful infection is likely achieved by effector proteins produced in the nematode esophageal gland cells and released into host plant cells. Here we show that the *Meloidogyne incognita* MSP18 esophageal gland cell protein is conserved in the *Meloidogyne javanica* and *Meloidogyne graminicola* species infecting rice. The *MSP18* gene was upregulated throughout all nematode parasitic stages in rice. Transient expression assays in onion cells suggest that MSP18 is addressed to the cytoplasm of the host cells. Overexpression of *MSP18* in rice enhanced *M. javanica* and *M. graminicola* reproduction, indicating that the MSP18 protein facilitates RKN parasitism. Transient expression assays in tobacco showed that MSP18 suppressed the INF1-triggered programmed cell

death, suggesting that MSP18 can interfere with the plant defense pathways. Data obtained significantly broaden our knowledge of molecular players contributing to nematode pathogenicity, and highlight MSP18 as a novel RKN virulence effector able to modulate host immunity.

Molecular interactions that influence virulence contributions of the IPI-O family of *Phytophthora infestans* effectors
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Phytophthora infestans, causal agent of potato late blight, is a destructive pathogen that is a frequently recurring problem worldwide. Several resistance genes exist in potato to counter against this pathogen, but the majority has been overcome after introgression into popular potato varieties. The *RB* gene, derived from *Solanum bulbocastanum*, has effector recognition specificity to members of the IPI-O family. Recognition of the IPI-O1 allele by *RB* elicits a hypersensitive resistance response while IPI-O4 can suppress this response. We have carried out several experiments to determine the virulence contributions of IPI-O1 and IPI-O4 during infection, and to identify host proteins involved in IPI-O recognition/suppression using co-immunoprecipitation and yeast two-hybrid. Our results indicate that both IPI-O1 and IPI-O4 contribute to *P. infestans* virulence, but their impact is influenced by the pathogen genotype. Protein interaction studies have identified both cytosolic- and membrane-localized host proteins that interact with IPI-O and will help to elucidate the function of these effectors in pathogen virulence. Together, we hope that our understanding of the function of the ubiquitous IPI-O effector will assist us in identifying or developing improved host resistance genes in potato.

Identification of genomic regions associated with host specificity and aggressiveness in *Ceratocystis* species
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The genus *Ceratocystis sensu stricto* includes more than 30 species of fungi that are pathogens of fruit and forest trees as well as agronomic crop plants. The type species, *C. fimbriata sensu stricto*, is host specific and causes black rot on *Ipomoea batatas*. *Ceratocystis manginecans* is genetically closely related to *C. fimbriata s.s.* and causes cankers and wilt on *Acacia* spp., mango and pomegranate, amongst others, but is not pathogenic to *I. batatas*. Despite their increasing economic importance, there is a paucity of knowledge regarding the factors that influence the host specificity and pathogenicity of these *Ceratocystis* spp. In this study, an interspecific cross was made between a *C. fimbriata* isolate from *I. batatas* and a *C. manginecans* isolate from *A. mangium*. Seventy F_1 progeny isolates were selected to investigate the inheritance of pathogenicity, mycelial growth rate and conidial production. Whole genome-based sequence analysis allowed construction of a linkage map that consisted of 467 SNPs, distributed across eight linkage groups and that spanned 1200 cM. We subsequently identified one highly significant QTL associated with growth rate on MEA, one associated with pathogenicity on *A. mangium* and two QTLs associated with pathogenicity on *I. batatas*. Candidate genes present in the QTLs are currently being characterized. These will be identified by investigating the presence/absence of genes in a species or nucleotide variations between the species.

Investigating host preference of *Acidovorax citrulli*, the causal agent of bacterial fruit blotch of cucurbits
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Acidovorax citrulli causes bacterial fruit blotch of cucurbits (BFB), and can be assigned to two groups: I and II. The association of group I and II *A. citrulli* strains with different cucurbit hosts strongly suggests host preference. We observed significant differences in pathogenicity between representative group I and II *A. citrulli* strains on watermelon and melon fruits, but not on foliar tissues. Thus, we hypothesized that *A. citrulli* host specificity occurs in cucurbit fruit, but not foliar tissues. The objective of this study was to assess differences in cucurbit host preference between group I and II *A. citrulli* strains under field conditions. This is important for understanding the factors that control virulence and to develop effective strategies to manage BFB. We planted a mixed plot with four cucurbit species in Tifton, GA USA and initiated a BFB outbreak with representative group I and II *A. citrulli* strains. We observed that 30%, 60%, 67%, and 71% of *A. citrulli*-positive watermelon, melon, pumpkin, and squash leaf samples were infected with the group I strain, respectively. However, 100% of the symptomatic watermelon fruit samples were infected with the group II strain, while 86% of symptomatic melon fruits were infected with the group I strain. These data support the hypothesis of *A. citrulli* host specificity in cucurbit fruits but not foliage under natural field conditions. Further studies will confirm and explore the nature of this host specificity.

Thioredoxinand glutaredoxin systemsrequired for oxidative stress resistance, fungicide sensitivityand virulenceof *Alternaria alternata*
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This study determines the function of thioredoxins and glutaredoxinsin the phytopathogenic fungus *Alternaria alternata*via analyzing mutants from the targeted deletion of genes encoding thioredoxin peroxidase (*Tsa1*), thioredoxin reductase (*Trr1*), glutathione reductase (*Glr1*) and glutathione synthetases (*Gsh1* and *Gsh2*). *Trr1*, *Glr1*, *Gsh1*, *Gsh2* but not *Tsa1* are required for growth and conidiation. Reduced growth and conidiation seen in the *Trr1* or *Glr1* deletion mutant can be restored by glutathione. Deletion mutants showing growth inhibition by oxidantsare defective for H_2O_2detoxification and induce smaller lesions on citrus leaves. *Trr1*, *Glr1* but not *Tsa1* also contribute to NaCl resistance. *Glr1* is required for sorbitol resistance and responsible for resistance to mancozeb, boscalid but not chlorothalonil fungicides, a novel phenotype that has not been reported in fungi. *Trr1* is required for resistance to boscalid and chlorothalonil fungicides while *Trr1* confers susceptibility to mancozeb. *Tsa1* deletion mutant displays wild-type sensitivity to test fungicides. The expression of *Tsa1* and *Trr1* is regulated by the oxidative stress responsive regulators Yap1, Hog1 and Skn7. The expression of *Tsa1* but not *Trr1* is also regulated by the NADPH oxidase. The results indicate that the capability to resist oxidative stressis required for virulence of *A. alternata*.

A genetic locus determining pathogenicity of *Pantoea ananatis*
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Pantoea ananatis (*Pa*) is a gram-negative plant pathogenic bacterium. *Pa* strains are divided into three groups (Group I, II and III) depending on their pathogenicity on rice, onion and melon and ability to induce hypersensitive response (HR)-like reaction in tobacco (Kido et al. 2010). The whole genome analysis and transposon-tagging analysis on one rice isolate SUPP2219 (Group I) suggested that a genetic region spanning ca. 19 kb in length existed exclusively in the known genomes of plant pathogenic *Pa* strains but not in saprophytic *Pa*. Tn-*5* insertion in some genes of this region abolished the virulence of the isolate. This genetic region was tentatively named as PASVIL (*Pantoea ananatis* specific virulence locus). PCR and Southern-hybridization tests revealed that PASVIL existed in all of Group I strains examined but not in Group II and III strains. In complementation tests, one large clone (pL422), spanning nearly entire region of PASVIL (ca. 18 kb), not only restored the virulence of the mutants, but also turned non-pathogenic Group III strains virulent. PASVIL contains at least 19 ORFs, coding membrane proteins, proteins for amino-acid transport and metabolism, and transposases, adjacent to tRNA-Phe gene at one border. The GC content of PASVIL was ca. 39%, significantly lower than that of whole genome average 53%. It was deduced that PASVIL might constitute a pathogenicity island, encoding pathogenicity determinant(s) not described before in *Pantoea* spp.

Grossi de Sa M., Petitot Anne-Sophie, Lisei de Sa M. E., Mezzalira I., Beneventi M. A., Amora D. X., Albuquerque E. V. S., Grossi de Sa M. F., Fernandez Diana. (2018)

Functional analysis of the MSP18 root-knot nematode virulence gene in rice

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