

symptomatic smooth blackberry (syn. Canadian blackberry, *Rubus canadensis* L.) displaying chlorosis, vein yellowing and leaf deformation reacted positively in RT-PCR when tested with universal primers for viruses belonging to the family Tymoviridae. Amplicons from all four samples were cloned and sequenced. Analyses showed high sequence conservation (96–99% identity) among clones from different specimens, indicating infection by the same virus in all tested samples. Computer analysis of the viral genome revealed the presence of a single, long open reading frame resembling the marafivirus genome. BLAST search showed that the virus present in tested samples is putatively a new member of the genus Marafivirus that shares ca. 55–60% common aminoacids with the polyproteins encoded by genomes of Citrus sudden death-associated virus (CSDaV), Maize rayado fino virus (MRFV) and Oat blue dwarf virus (OBDV). Virus-specific primers were designed in order to study the etiological role of this virus in the disease and its incidence in wild and cultivated *Rubus* spp.

An undescribed dsRNA virus from *Rhododendron*

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Foliar samples of *Rhododendron maximum* L. (Great Rhododendron, Great Laurel, Big Rhododendron) were collected at different locations in Great Smoky Mountain National Park and assayed for the presence of RNA viruses by dsRNA analysis. A dsRNA molecule of an estimated size of 3.5 kbp was detected in some of the samples, most of them without disease symptoms. Purified dsRNAs were used as a template for cloning and sequencing. Sequence data indicated that the dsRNA represents the genome of a novel plant virus for which the name Rhododendron virus A (RhVA) is proposed. The genome consisted of ca. 3,430 bp and contained two partially overlapping open reading frames (ORFs). Both ORFs were phylogenetically related with orthologous genes present in the recently described Tomato yellow stunt-associated virus and Blueberry fruit drop virus. The three viruses clustered together forming an independent clade among dsRNA viruses implying that they may belong to a yet-to-be-established taxon of phytoviruses. PCR tests using ITS-specific primers did not reveal the presence of an endophytic fungus. This confirms that rhododendron is the host for this new dsRNA virus.

Detection and identification of an umbravirus from *Ageratina altissima*

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Random-primed cloning of reverse transcribed dsRNAs extracted from white snakeroot (*Ageratina altissima* L. King & H. Rob.) specimens showing chlorotic spots and mosaic symptoms yielded several clones of phytoviral origin. Comparison of initial data with the sequences available in GenBank showed that the virus had a close relationship with members of the genus Umbravirus. The complete genome of this virus showed the presence of four open reading frames (ORFs), the genomic organization resembling that of umbraviruses. ORFs 1 and 2 are likely translated via a -1 frameshift mechanism as a single polypeptide involved in viral replication, whereas 3'-proximal overlapping ORFs 3 and 4 enable cell-to-cell and long distance movements within the plant. Viral polymerase was closest to Groundnut rosette virus (65% identical residues), while proteins encoded by ORF3 and 4 showed 30–40% identities with orthologous products of Pea enation mosaic virus-2 (PEMV-2) and Tobacco mottle virus (TMOV) respectively. According to the overall molecular data, the virus from *Ageratina* is likely an undescribed member of the genus Umbravirus for which the name *Ageratina chlorotic spot virus* (AgCSV) is proposed. As umbraviruses rely on a specific helper virus for transmission by aphids, the research on identification of a virus associated with AgCSV is currently on-going.

Genotypic analysis among Iranian isolates of *Cercospora beticola*

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Cercospora beticola is the causal agent of cercospora leaf spot on sugar beet and has a large negative impact on the yield and quality of sugar beet production worldwide. In this study, genotypic analysis among twenty four isolates of *Cercospora beticola* from different regions of Iran was investigated using restriction fragment length polymorphism of the internal transcribed

spacer (ITS-RFLP) and RAPD-PCR analyses. Fourteen decanucleotide primers were selected for RAPD analysis. The results of RAPD revealed a wide DNA polymorphism between Iranian isolates of *C. beticola* and clustered all isolates into nine groups. There was a clear relationship between cluster grouping and geographical origin. The restriction pattern of internal transcribed spacer of rDNA (ITS1-5.8-ITS4) was investigated by using three restriction endonucleases *Eco*R1, *Taq*I and *Bsu*R1. The undigested DNA fragment length of all isolates was estimated 550bp and no rDNA polymorphism was observed after digestion with endonucleases *Eco*R1 (280, 270 bp), *Taq*I (330 bp) and *Bsu*R1 (240, 220, 90 bp). According to these results, RAPD marker is suitable for showing the highest level of genetic variation. On the other hand, ITS-RFLP shows the highest level of similarity and confirms the identification of *Cercospora* spp. These results represented the first documentation of using ITS-RFLP and RAPD for Genotypic analysis among Iranian isolates of *Cercospora beticola*.

In planta distribution and quantification of Asiatic strain of citrus Huanglongbing pathogen

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Huanglongbing is one of the most devastating diseases caused by an uncultured phloem limited bacterium, *Candidatus Liberibacter* spp. In this study, a combination of traditional and real-time PCR targeting the putative DNA polymerase and 16S rDNA sequence, respectively, were used to examine the distribution and movement of the Asiatic strain (Las) of citrus Huanglongbing in the infected citrus tree. We found that Las was distributed in bark tissue, leaf midrib, roots, and different floral and fruit parts, but not in endosperm and embryo, of infected citrus trees. In addition, quantitative real-time PCR was used for quantification of the Las in citrus leaf midribs, roots, periwinkle leaves and psyllids. Quantification analysis of the HLB bacterium indicated that Las concentrations varied widely among different tissues of the citrus tree. A relatively high concentration of Las was observed in fruit peduncles. Our data from greenhouse infected plants indicated that Las was systemically transmitted from infection site to different parts of the plant. Our study also indicated that a minimum bacterial concentration is required for HLB symptom development. Understanding the distribution and movement of the HLB bacterium inside an individual citrus tree is critical for discerning its virulence mechanism and to develop management strategies for HLB.

Survey of huanglongbing (HLB) and citrus canker in the Rio Grande Valley

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Huanglongbing (HLB) and citrus canker are the two most dreaded diseases for citrus production in the Rio Grande Valley (RGV), Texas. The objective of this study was to detect as early as possible these diseases in the RGV and thus to mitigate the tremendous economic losses that can be inflicted on the citrus industry. Within each one mile square quadrat of the RGV, one to four citrus trees have been strategically selected and used routinely to monitor populations of the Mexican fruit fly (*Anastrepha ludens*). Presently, these trees are also being used as sentinel trees to detect HLB and citrus canker. Before the survey, fruit fly trappers from the Texas Department of Agriculture were trained on the recognition of HLB and citrus canker and, thereafter, asked to collect diseased citrus leaves in addition to their normal duties. During years 2006 and 2007, a total 314 and 1685 citrus leaf samples were collected, respectively. The majority of leaf samples were from grapefruit and less from orange. Regardless of host, most samples were from groves and less from dooryards. Samples were examined in the laboratory for symptoms of HLB and citrus canker. Citrus trees showing symptoms of Zn deficiency, yellowing or lop sided fruit were revisited. Other foliar diseases or insect damage were also recorded. None of the citrus leaves or fruits examined showed diagnostic symptoms of HLB or citrus canker. However, a follow up survey is suggested of trees showing symptoms of Zn deficiency (2006 = 15%; 2007 = 8%), mosaic or yellowing (2006 = 3%; 2007 = 7%), or psyllid attack (2007 = 5%). Greasy spot (*Mycosphaerella citri*), Fe deficiency, and Mg deficiency were the most frequent diseases found in this survey. Close monitoring of HLB and citrus canker throughout the Valley must continue.

Species of *Fusarium* associated with the rhizosphere-soil of *Arundo donax* in Laredo-Texas

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Giant reed (*Arundo donax*), has formed dense thickets and is quickly spreading and expanding in its range along the Rio Grande River in Texas and