Detection, Identification and Characterization of New and Emerging Viral and Bacterial Diseases of Ornamental Plants

PRINCIPAL INVESTIGATORS:
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PROJECT OBJECTIVES:
1. Characterize viruses of major significance to ornamental and nursery crops, including "new" currently uncharacterized or emerging viruses affecting key ornamental crops. **2.** Determine the genome organization of selected important ornamental viruses and develop full-length infectious clones to determine the genes involved in pathogenicity. **3.** Develop improved tools and evaluate methodologies for the identification, detection, and control of bacterial diseases of major significance to woody and floral ornamental crops.

ACCOMPLISHMENTS:

1. Produced virus-specific reagents (PCR primers and/or antisera) for detection and identification of several new and/or emerging viruses infecting ornamental plants. These include specific PCR primers for: *Alternanthera mosaic virus* (AltMV; affecting multiple ornamentals), *Angelonia flower break virus* (AnFBV; angelonia, hybrid phlox, verbena), *Bacopa chlorosis virus* (BaCV; bacopa), *Cucumber mosaic virus* (CMV; gladiolus qRT-PCR), *Freesia sneak virus* (FreSV; lachenalia and freesia – detected for first time in the United States), *Impatiens flower break virus* (IFBV; New Guinea Impatiens), *Ligustrum necrotic ring spot virus* (LNRSV; first report in impatiens), *Lily virus X* (LVX; Lily, Tricyrtis), *Lolium latent virus* (LoLV; ryegrass), *Pepper mild mottle virus* (PMMoV; ornamental peppers), *Phlox virus M* (PhlVM; hybrid annual phlox), *Phlox virus S* (PhlVS; creeping phlox), *Phlox virus B* (PhlVB; sweet william phlox), *Tricyrtis virus Y* (TrVY; Tricyrtis), a 'new' carlavirus in Salvia, and the pansy ilarvirus (pansy).

   Also produced broad-spectrum primers for the detection of potexviruses and carlaviruses. Produced antisera for detection of LoLV, PhlVS, and *Pelargonium line pattern virus* (PLPV). Because we had been unable to obtain sufficiently purified BaCV for antiserum production, we have now cloned the coat protein gene into a bacterial expression system to produce virus-specific antiserum to be used for routine detection. A novel carlavirus, *Phlox virus M* (PhlVM) was detected in commercial hybrid (annual) phlox, and more recently in mixed infections with either AnFBV or AltMV, causing more severe infections. Purified PhlVM directly from hybrid phlox, as no other host has yet been identified, and are also expressing PhlVM coat protein in a bacterial system for production of virus-specific antiserum. Determined partial sequences of one or more isolates of: PhlVM (carlavirus); *Columbian datura virus; Freesia mosaic virus; Omphalodes virus Y; Ornithogalum mosaic virus; Ornithogalum necrotic mosaic virus; Ornithogalum stripe mosaic virus; Spiranthes mosaic virus 3; Vallota mosaic virus* (potyviruses); and FreSV (ophiovirus).

2. Multiple infectious clones of AltMV have identified three viral genes affecting pathogenicity, and genes affecting systemic movement; substitution of the coat protein of a mild portulaca isolate eliminated necrosis. An infectious clone of LoLV has shown the importance of the

coat protein in systemic movement. An infectious clone of *Pelargonium chlorotic ring pattern* has been developed and is under evaluation. Full genome sequences of the carlaviruses PhlVS, PhlVB, the closterovirus *Carnation necrotic fleck virus*, the pelarspoviruses PLPV, *Pelargonium ringspot virus* and *Elderberry latent virus*, and the potyvirus IFBV have been determined, as well as almost complete sequences of BaCV (ilarvirus), *Carnation yellow fleck virus* (closterovirus), and FreSV (ophiovirus).

3. Developed extraction methods for nucleic acids from multiple types of plant material, for effective PCR amplification and identification of pathogens. Developed primers for detection, identification, and differentiation of specific isolates of *Xylella fastidiosa*, including oleander isolate. Detected *X. fastidiosa* in asymptomatic nursery stock of crapemyrtle, Japanese stewartia, Schipka laurel, and Japanese flowering cherry. Utilized broad-spectrum and oleander strain-specific PCR to differentiate an oleander isolate from other isolates. Determined that *Ralstonia solanacearum* R3b2 does not infect begonia, Browallia, Impatiens, pansy, snapdragon, verbena, Dusty miller, poinsettia, sage, and oxalis are not hosts of *R. solanacearum* R3b2, but that portulaca is infected following soil drench inoculation. Determined susceptibility of geranoium cultivars to *R. solanacaerum*. Showed that clove oil has significant antibacterial action *in vitro* on *Ralstonia*, *Xanthomonas*, *Erwinia*, *Pseudomonas*, *Streptomyces*, *Agrobacterium*, and *Rhodococcus*, and demonstrated that it is effective as a pre-plant soil fumigant to control bacterial wilt of geranium and tomato caused by *Ralstonia*, without phytotoxicity under greenhouse conditions.

TECHNOLOGY TRANSFER/IMPACT:

Submitted summaries of research on viruses of ornamentals for the Ornamentals Virus Discussion Group of other researchers and industry personnel at the annual American Phytopathological Society (APS) meetings; assisted nurseries by identifying viruses in ornamental plants, and transferred serological reagents and nucleic acid sequence information for development into diagnostic kits and technologies, to both nurseries and diagnostic companies. Also identified virus-free plant material for future propagation. Made presentations at scientific (APS; and International Symposium on Virus Diseases of Ornamental Plants) and industry (Ohio Short Course) meetings, and industry publications (American Nurseryman) on new viruses and means of detection and identification. Worked with Agdia, Inc., to assist in development of improved diagnostic reagents, to validate detection protocols, and to help determine the identity of potential 'new' or 'unknown' viruses. Currently developing (under an NRI Plant Biosecurity program grant) a Universal Plant Virus Microarray for detection and identification of both characterized and uncharacterized viruses. Gearing up to produce cross-reactive monoclonal antibodies for broad spectrum detection of additional virus groups, to follow up on our highly cross-reactive antibody for detection of potyviruses (used in Agdia's Potyvirus group test). Deposited, released, and/or updated viral, bacterial, and mouse antibody sequence data in GenBank.

ADDITIONAL FUNDING/EXTERNAL SUPPORT:

Permanent FNRI funding established the Bacteriology position held by Dr. Qi Huang. Temporary FNRI funding (FY 2002 to FY 2008) supported virus identification and diagnostics research. CRADA and Trust Fund agreements with Agdia, Inc. support similar work on

detection and diagnosis of specific viruses. We (John Hammond/Ramon Jordan) also have an NRI Plant Biosecurity program grant to develop a Universal Plant Virus Microarray for detection and identification of any plant virus, in collaboration with several other institutions. Healthy plant material has been provided by EuroAmerican.

COLLABORATORS:

Agdia**,** Inc, and anonymous Agdia customers; Jan Hall (Target Specialty Products; formerly with Ecke Ranch); Fred Ceballos/Sharon Kavanaugh (EuroAmerican); Wendy O'Donovan (Ecke Ranch); Deborah Mathews (University of California-Riverside); Melodie Putnam (Oregon State University); Anna Maria Vaira (IVV, CNR, Italy); Karen Rane (University of Maryland); Mary Ann Hansen (Virginia Tech); Dennis Lewandowski (Ohio State University); Scott Adkins (ARS, Fort Pierce, Florida), Caitilyn Allen (University of Wisconsin); David Norman (University of Florida); and Kathy Kamo and Dilip Lakshman (ARS FNPRU, Beltsville, Maryland). Universal Plant Virus Microarray collaborators include Claude Fauquet (Danforth Center); David Wang (Washington University); Kael Fischer (University of Utah); Keith Perry (Cornell University); and Ulrich Melcher (Oklahoma State University).