Sensitive Detection of Single and Mixed Viral Infections in Ornamental Plants at All Stages of the Propagation Cycle

PRINCIPAL INVESTIGATOR:
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PROJECT OBJECTIVES:
Detection of viruses in ornamental plants is particularly important at the propagation level since infected mother plants can be responsible for the dissemination of 1,000's of infected cuttings. In addition, viruses that cause mild or even no symptoms in their original ornamental hosts under greenhouse conditions, may cause severe symptoms in other varieties or species, including food crops, once plants become established in the landscape. New viruses are being found every year, most of which come into the United States through the movement of plant material from off shore locations. As new plant varieties are produced and distributed, their associated viruses come with them. These new viruses may escape detection since most routine screening relies on specific antibody or nucleic acid based tests for known viruses, and because symptoms may be absent or quite subtle, especially in young plantlets. Once discovered, new viruses can trigger state quarantines of plant products, disrupting the industry's ability to conduct business.

My program focuses on improving the detection of known plant viruses in single and mixed infections as well as characterizing new viruses in order to develop methods for their detection, assess their threat to agriculture, and to provide information to regulatory agencies to help avoid or reduce quarantines. Similar work with viroids is also being implemented.

ACCOMPLISHMENTS:
We have determined that strains of *Nemesia ring necrosis virus* (NeRNV) found in California and elsewhere in the United States are distinct from those found in Europe and care must be taken if using RT-PCR for detection since primers developed for the European strain do not work with most California strains. Primers were developed which react with all known strains of NeRNV.

A survey for the presence of *Angelonia flower break virus* (AnFBV) was conducted in California. AnFBV is a recently discovered plant virus that was found to be present in several states. Samples were collected from retail and wholesale locations in Riverside, Sacramento, San Diego and Santa Barbara counties. Sixty four percent of the plants (Angelonia, Nemesia, and Verbena) were positive for AnFBV with at least one positive plant being found in each of the four counties. AnFBV isolated from Nemesia and Verbena plants were most similar to strains found in Israel and Florida, while the AnFBV from Angelonia were most similar to Maryland strains. This variation implies that multiple introductions of AnFBV into California have been made. This data was provided to the California Department of Food and Agriculture along with host range data published by other researchers to show that AnFBV is not a threat to California agriculture and is already established in the state. No quarantine was issued for AnFBV.

One major ongoing research objective is to study the timing and methods used for virus detection after plants have undergone meristem isolation using tissue culture. Our previous research showed that instead of fully eliminating viruses using tissue culture, they could simply be reduced to non-detectable levels, resulting in negative tests early on in the plant's growth cycle. However, as the plants developed over time, virus levels increased and could again be detected, sometimes after large scale propagation had begun. Verbena was chosen as the host plant for this study due to the fact that it is commonly infected with multiple viruses which may be the result of the above scenario of viruses escaping detection after tissue culture. Thus far we have developed a robust tissue culture system for verbena by testing eight different media, then selecting the best three for regeneration trials with three different varieties of verbena to ensure broad future application. One medium was selected as satisfactory for each verbena variety. Verbena plants infected with NeRNV were used for the removal of shoot tip meristems 0.3-0.5 mm in length, which were placed on the medium and allowed to grow for 3-6 weeks until enough tissue was available for removal without damaging the plantlet, then were tested weekly after that by ELISA, RT-PCR, and inoculation to *Nicotiana benthamiana*. Thirty two percent of the plants tested positive for NeRNV at the first screen and were removed from the trial. Seven percent of the plants were negative for NeRNV at their first test date, then were positive the next week. A single plant had 2 weeks of negative tests before testing positive the third week. Tests on the remaining 33 plants are continuing. ELISA and RT-PCR have been equal in sensitivity so far. This trial will be repeated with NeRNV and also with AnFBV and mixed infections of both viruses. Previous work has shown that the presence of mixed infections can affect the detection of one or more viruses in the complex.

TECHNOLOGY TRANSFER/IMPACT:

This work has, and will, resulted in the adaptation of virus testing protocols both at commercial and private in-house labs. In addition, my research has prevented state quarantines from being imposed on plant products. I regularly share results with a major plant propagation facility in southern California as well as present my data each year to the Ornamental Virus Discussion Group at the American Phytopathological Society (APS) meeting. I was invited to give lectures about my work in a special Symposium at the APS meeting in 2007 and at the Society of American Florists Pest Management Conference (SAF-PMC) in 2009, each with audiences of approximately 200.

Two publications have recently been produced (see below) with 2 full papers in preparation. I also wrote articles for the Proceedings of the SAF-PMC meeting and the Winter 2009 issue of the California Ornamental Research Foundation newsletter.

Mathews, D.M. and Dodds, J.A. 2007. Detection and characterization of a new Tymovirus in ornamental plants. Phytopathology 97:S131 (abstract).

Mathews, D.M. and Dodds, J.A. 2008. First report of Angelonia flower break virus in Nemesia spp. and other ornamental plants in California. Plant Disease 92:1263.

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COLLABORATORS:
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