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First Confirmed Report of *Tobacco ringspot virus* in Cucurbits Crops in Oklahoma

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Cucurbits are major cash crops of vegetable growers in Oklahoma, particularly watermelon, which is the official state vegetable. In 2010, during a survey for cucurbit viruses (1), symptomatic leaf samples of cucumber (*Cucumis sativus*), cantaloupe (*Cucumis melo*), pumpkin, (*Cucurbita pepo*), squash (*Cucumis maxima*), and watermelon (*Citrullus lanatus*) showing mild to severe mosaic, mottling, and chlorotic spots were collected in Atoka, Blaine, Jefferson, and Tulsa counties. A total of 161 samples were tested by dot-immunobinding assay (DIBA) (2) using *Tobacco ringspot virus* (TRSV; genus *Nepovirus*, family *Comoviridae*) specific antiserum. Fourteen samples of cantaloupe, pumpkin, and watermelon from Blaine, Jefferson, and Tulsa counties were positive serologically to TRSV. At least one to two samples from each representative cucurbit collected in the field above were used as a source for mechanical inoculation. Sap was extracted from symptomatic leaves using 0.1 M K_2HPO_4 buffer (pH 7.2) and rub-inoculated to two squash (cv Elite) seedlings at cotyledonary stage pre-dusted with Carborundum. Seven to 10 days post-inoculation, all inoculated plants produced typical TRSV symptoms including chlorotic spots, systemic ringspot, severe leaf deformation, mottling, and stunting. Sap and total RNA was extracted from 10 mechanically inoculated squash seedlings and tested by DIBA and reverse transcription (RT)-PCR using specific TRSV primers (F: 5′-TACAGTGAGGATGCATG-3′ and R: 5′-AGTAGCTGCGACAAGCCA-3′). All of the tested samples were positive by DIBA except the negative control. Similarly, all samples from mechanically inoculated plants were also positive by PCR showing the expected 1,039-bp PCR product when analyzed by agarose gel electrophoresis. Total RNA obtained from mock-inoculated squash seedlings used as a control was negative by PCR. Amplified PCR product (1,039 bp) was directly sequenced from three infected squash seedlings. Sequence analysis confirmed that the virus shared 90 to 92% nucleotide and 94% amino acid identities with RNA2 of TRSV isolate from the U.S. (Accession No AY363727) available in the GenBank database. Total RNA extracted from original tissues of 14 DIBA positive samples collected from field were also positive by RT-PCR. The presence of TRSV could pose a serious threat to many vegetable crops, particularly cucurbits and other agricultural crops, due to its wide host range (3). This report confirms the suspected occurrence of TRSV in 1956 from watermelon in Oklahoma (4).

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