New Disease Reports

Necrotic streak disease of tomato in Florida caused by a new ilarvirus species related to *Tulare apple mosaic virus*

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During surveys for the emerging tospoviruses *Tomato chlorotic spot virus* (TCSV) and *Groundnut ringspot virus* (GRSV), virus-like necrosis of leaves, petioles and stems (Fig. 1), and necrotic rings or spots on fruits (Fig. 2) of fresh market tomato plants were observed in south (Miami-Dade County) and southeast (Palm Beach County) Florida beginning in October 2013. Incidence of plants with symptoms was generally less than 3% but in some fields was considerably higher. More than 1000 plants were rogued on a single farm in Miami-Dade County during the fall 2013 season. Total RNA was extracted from representative samples using a RNeasy Plant Mini Kit (Qiagen, Valencia, CA) and tested for TCSV, GRSV and *Tomato spotted wilt virus* by RT-PCR as previously described (Webster *et al*., 2015). When no products were amplified, nine virus/viroid genera or groups, and an additional ten virus species including the ilarviruses *Tobacco streak virus* (TSV) and Tomato necrotic spot virus (ToNSV; Batuman *et al*., 2009) were assayed by RT-PCR and/or DAS-ELISA (Agdia, Inc., Elkhart, IN) all with negative results. Light microscopic examination for inclusion bodies revealed no known virus-induced structures. Symptomatic tomato plant tissue was used to mechanically inoculate tomato plants, and symptoms of leaf, petiole and stem necrosis similar to those observed in the original field samples developed in the inoculated tomato plants.

Ultimately, a 412 bp fragment (GenBank Accession No. KP861233) was amplified from total RNA by RT-PCR using ilarvirus primers provided by Agdia, Inc. Sequence analysis indicated that the nucleotide (nt) and deduced amino acid (aa) sequences of the fragment were 68 to 80% and 67 to 83% identical, respectively, to RNA1 and the replicase protein 1a of subgroup 2 ilarviruses with considerably less identity to other previously reported subgroup 1 tomato-infecting ilarviruses including TSV (54% nt, 43% aa) and *Parietaria mottle virus* (49% nt, 46% aa; Galipienso *et al*., 2005). Thus, primers were designed from conserved regions of available RNA3 sequences of subgroup 2 ilarviruses [*Asparagus virus 2*, *Citrus leaf rugose virus*, *Citrus variegation virus*, *Elm mottle virus*, *Spinach latent virus* and *Tulare apple mosaic virus* (TAMV)] and used to amplify the movement protein (MP) and coat protein (CP) genes. Sequence analysis showed that the MP and CP genes (KP861234 and KP861235) were most closely related to, but distinct from, TAMV at 79 and 83% identity, respectively. Deduced MP and CP aa sequences were 76 and 77% identical, respectively, to those of TAMV. These results indicate that the tomato symptoms initially observed in Florida in fall 2013, and subsequently in spring and fall 2014, are caused by a new subgroup 2 ilarvirus species, for which the name Tomato necrotic streak virus (TomNSV) is proposed. Potential TomNSV transmission by pollen, seed and/or insect vectors that may include thrips similar to other ilarviruses (Sdoodee & Teakle, 1987; Aramburu *et al*., 2010) is not currently known and requires additional research.

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Figure 1

Figure 2

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