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Trichoderma Rot on 'Fallglo' Tangerine Fruit in Florida

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In Sept. 2009, trichoderma rot was observed on 'Fallglo' tangerine fruit after harvest, degreening, and storage under ambient conditions. The decay symptoms were as previously reported for trichoderma rot. The fungus was isolated from the diseased peel of symptomatic fruit and its morphological characteristics were typical of *Trichoderma*, as described previously. The fungus was identified as *Trichoderma atroviride* based on morphology and confirmed by sequencing comparison to known isolates. 'Fallglo' fruit developed the same disease symptoms after being wound-inoculated with a spore suspension of *T. atroviride* (2.1×10^7/mL) isolated from the original diseased tissue. This is the first report of *T. atroviride* being pathogenic to citrus fruit.

Postharvest decay of fresh citrus is a major cause of product losses after harvest (Wardowski et al., 2006). Diplodia stem-end rot (*Lasiodiplodia theobromae*), green mold (*Penicillium digitatum*), brown rot (*Phytophthora citrophthora*), and early season anthracnose (*Colletotrichum gloeosporioides*) are among the most important diseases of Florida citrus. Trichoderma rot of citrus is caused by *Trichoderma viride* Pers. ex Gray (syn. *T. lignorum* Tode ex Harz) (Eckert and Brown, 2000). It was reported in California in 1925 (Fawcett, 1925) and later reported to cause severe losses to export shipments from California during the 1950s and 1960s (Harding and Savage, 1962). The disease was rated as the second most important disease after green mold in South Africa (Christ, 1964). In Florida, *Trichoderma* has been mentioned only in passing as a potential source of decay on Florida citrus (Smoot et al., 1983). While not anticipated to be a significant source of decay of Florida citrus, this manuscript identifies a different species of trichoderma rot occurring on Florida 'Fallglo' tangerine that has not been reported on citrus before.

Materials and Methods

Source of fruit. Trichoderma rot was found on stored 'Fallglo' fruit harvested 24 Sept. and 5 Oct. 2009 from a commercial grove located in Indian River County. The trees were managed using commercial fresh fruit cultural practices and were used as part of a preharvest fungicide study. The fruit had been dipped with water or one of seven compounds, mostly fungicides, and then harvested 3 or 14 d afterwards. After harvest, the fruit were degreened for 5 d with 5 ppm ethylene at 29 °C and ~95% relative humidity (RH). After degreening, the fruit were stored under ambient room conditions (~23 °C and 60% RH) and evaluated weekly for decay and peel disorders. After 35 d of storage, when up to 68% of the fruit had developed diplodia stem-end rot (*Lasiodiplodia theobromae*), small amounts (~3%) of an unknown decay symptom appeared on some of the fruit (Fig. 1). There was no relationship between the development of this unknown decay and the fungicide treatments, likely because of its variable and low occurrence compared to the amount of fruit lost to diplodia. Control fruit that had been dipped in water prior to harvest and that developed the unusual decay symptoms were placed into a plastic container with >95% RH to further observe symptom development.

Isolation and purification. Diseased peel tissue from symptomatic fruit was cut into small (~5 mm^2) pieces using a sterile knife and pieces were surface sanitized with 6% sodium hypochlorite solution (The Clorox Company, Oakland, CA) for 45 s and then rinsed three times with sterile, deionized (DI) water. The tissue samples were placed into 100 × 15 mm petri

Fig. 1. 'Fallglo' tangerine fruit with trichoderma rot after 35 d of storage at about 23 °C.

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dishes containing potato dextrose agar (PDA; Becton, Dickinson and Co., Sparks, MD) medium and incubated at 25 °C. Mycelia growth was observed for more than 14 d. Pure cultures of the isolates were obtained by transferring the colonies to PDA plates via conidial transfer and were used to identify the fungal genus (Kiraly et al., 1974).

Fruit inoculation. Five healthy 'Fallglo' fruit from the same block were harvested and held at 10 °C for 35 d before transfer to room temperature 2 h before surface disinfection with 75% alcohol. The fruit were allowed to air dry and then each fruit was punctured at two sites along the equator using a sterile needle (28-G1/2, Becton Dickinson and Company, Frankin Lakes, NJ), and approximately 5 µL of spore suspension from a 10-day-old culture (2.1 × 107 CFU/mL) were placed on the surface of the wounded sites. The fruit was immediately placed into a covered plastic tray with high humidity maintained by placing a sterile wet rag on the bottom of the tray, and kept on the lab bench at about 24 °C. Two healthy fruit, which were wounded but not inoculated, were included in the same tray as a negative control.

Species identification. The original fungal culture was used to create new plates on PDA amended with chloramphenicol (177 mg/L) and streptomycin (263 mg/L) (PDA-SC). Hyphal tips were excised from the thallus and placed in a modified Van Tieghem cell (Tuite, 1969). The hyphal-tip culture was used to inoculate potato dextrose broth (PDB) for DNA analysis and PDA plates to characterize the fungus morphologically. The fungus was grown at 25 °C with 12 h diurnal light provided by a bank of standard 34-W cool-white fluorescent bulbs. Morphological data was submitted to the USDA, PEET Project Trichoderma interactive key (Samuels et al., 2011).

For the DNA sequence analysis, one 5-mm plug was taken from the edge of the actively growing mycelium and placed in 50 mL of PDB. The mycelial mat from a 3-day-old culture was ground in liquid nitrogen with a mortar and pestle. Genomic DNA was extracted using the UltraClean™ Microbial DNA Isolation Kit (MO-BIO Laboratories, Carlsbad, CA). The internal transcribed spacer region was amplified using primers SR6 and LR1 (Abadi, 2008; Vilgalys and Hester, 1990). Amplification of a portion of the translation elongation factor 1α (EF1-α) was performed using primer pair EF-728F and Tef1R (Hanada et al., 2008). All reactions were performed in a PTC-200 MJ Research thermocycler (Waltham, MA) with 2xGoTaq (Promega, Madison, WI). The amplification products were gel electrophoresed (1% agarose) and extracted from the gel using the QIAquick® gel extraction kit following the manufacturer's instructions (Qiagen Sciences, Gaithersburg, MD). DNA concentration in the extracted products was estimated using a ND-1000 spectrophotometer (Nanodrop Technologies, Inc., Wilmington, DE) and extracted amplicons were sequenced using primers for amplification on an ABI3730XL automated sequencer at the USHRL DNA Sequence Support Laboratory. DNA sequences were edited to remove ambiguous bases using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI) and were submitted to TrichOKEY 2 (Druzhinina et al., 2005) and TrichoMARK (Kopchinskiy et al., 2005) for analysis.

Results and Discussion

Symptoms and species identification. Trichoderma lesions on 'Fallglo' tangerine that developed after 35 d storage under room conditions and high RH were as described by Eckert and Brown (2000) (Fig. 1). Morphological characteristics that were evident in isolation plates were typical of *T. atroviride* as described by Barnett and Hunter (1998). Koch's postulates were performed on healthy fruit using the organism isolated from original fruit disease lesions; symptoms and fungal morphology that developed on the inoculated fruit were identical to the initial decay. Control fruit that were wounded but not inoculated did not develop decay.

Amplification of the rDNA resulted in an amplified sequence of approximately 1180 bp. Four of four anchors were identified by TrichoMARK for ITS1 and 2. The informative portion of this sequence, containing the anchor sequences, was compared to the sequences in the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (ISTH) database and had 100% identity to the *Hypocrea atroviridis/T. atroviride* isolate CBS142.95 (GenBank accession AY380906). Amplification of the partial sequence of the elongation factor 1-α resulted in a product of approximately 640 bp. A BLAST nucleotide collection search using the megablast option resulted in 100% identity of the putative citrus *T. atroviride* isolate with *T. atroviride* strain GJS 02-96 (GenBank accession DQ307550). These three combined pieces of information result in the conclusion that the isolate is *T. atroviride.*

Decay of Florida citrus fruit has likely has been infrequently caused by *Trichoderma* spp. since the early days of its production (Smoot et al., 1983). However, specific reports positively identifying *Trichoderma* as the causal organism on Florida citrus have not been found. This manuscript reports that the decay of Florida 'Fallglo' tangerine fruit was caused by *T. atroviride* P. Karsten. This species has not been reported as causing decay on citrus before. However, trichoderma rot is expected to remain a rare occurrence in Florida and should be easily controlled by careful handling, good sanitation practices, and the use of postharvest fungicides (Eckert and Brown, 2000; Pelser, 1972). Rapid cooling and holding fruit below 10 °C also slows the development of *Trichoderma* (Eckert and Brown, 2000)

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