OPEN ACCESS Freely available online

Journal of Citrus Pathology

Research Article

First report of citrus dry root rot caused by *Fusarium solani* on sour orange rootstock in Texas.

M Kunta[1*], B Salas[2], M Gonzales[1], and JV da Graça[1]

[1] Texas A&M University-Kingsville Citrus Center, 312 N International Blvd, Weslaco, TX 78599; [2] USDA-APHIS-PPQ-CPHST, 22675 N Moorefield Rd, Edinburg, TX 78541.

*Correspondence to: madhura.kunta@tamuk.edu

Citation: Kunta M, Salas B, Gonzales M, da Graça JV. 2015. First report of citrus dry root rot caused by *Fusarium solani* on sour orange rootstock in Texas. J Cit Pathol. iocv_journalcitruspathology_27974.

Abstract

A grapefruit tree on sour orange rootstock at a residential property in Mission, Texas, was suspected to have citrus dry root rot disease based on symptoms. The putative causal organism was isolated from infected roots and based on cultural and microscopic morphology and PCR, the pathogen was confirmed to be *Fusarium solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen. A total of 10 healthy sour orange rootstock seedlings were inoculated using conidial suspensions of the fungus by the standard root-dip method. Nine months after inoculation, the pathogen was re-isolated from symptomatic root and stem sections. Affected plants were stunted and displayed the classical symptoms of dry rot. The fungal colonies were confirmed to be *F. solani* based on fungal morphology and PCR. This is the first report of *F. solani* infecting sour orange rootstock plants in Texas.

Keywords: Citrus, *Fusarium solani*, Texas, roots, sour orange rootstock, dry root rot disease

Introduction

Dry root rot of citrus has been reported from California, Australia, and South Africa, usually for trees on trifoliate citrus or citrange rootstocks (Broadbent 2000). Apparently healthy trees suddenly wilt and die, and the roots are blackened and rotted with a brown, vascular discoloration within the stem of the rootstock. In Florida, similar decline symptoms occur with trees affected by blight and tangerine collapse, both with unknown etiologies (Graham et al. 1985). Various fungi have been isolated from trees affected with dry root rot, including *Coprinus micaceus* and *Diaporthe citri*, but *Fusarium solani*, a ubiquitous fungus with varying pathogenicity to citrus under stress conditions, can also induce dry root rot (Broadbent 2000). *F. solani* has also been isolated from affected citrus in Florida, and can cause root necrosis in trifoliate orange seedlings having depleted starch reserves. However, this fungus is not believed to be the primary cause of blight, which is considered to differ from tangerine collapse (Graham et al. 1985).

Dry root rot has not been reported previously in Texas, where most trees are grafted on sour orange rootstocks. However, the recovery of *Fusarium* spp. from both healthy and dead grapefruit twigs has been reported (Okamura and Davis 1987). In late March 2014, a homeowner in Mission, in the Lower Rio Grande Valley of Texas, submitted root samples (Fig. 1) from a diseased, uprooted grapefruit tree on sour orange rootstock to our diagnostic laboratory at A&M University Kingsville Citrus Center, Weslaco, Texas. Because roots on this tree displayed symptoms of light purple, vascular discoloration, we suspected that the tree had dry root rot. This paper reports on the results of fungal isolation, pathogenicity tests, and identification of the pathogen.

Materials and Methods

Fungal isolation

The roots were rinsed in running tap water to remove soil and debris, and tissue pieces (3 by 6 mm) showing vascular discoloration were excised and placed in Histosette II Biopsy Cassettes for surface sterilization. Tissue pieces were then sterilized in 70% ethanol for 30 s, and in 10% commercial chlorine bleach for 2 min, rinsed 3 times in sterile water, and air-dried under laminar flow. Sterilized tissue pieces were placed on culture plates with Komada's medium (Komada 1975). All culture plates were incubated in darkness at 28 °C for 10 to 15 days.

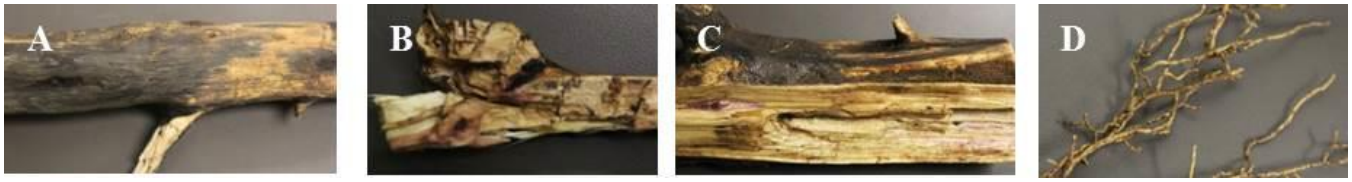

Fig. 1. Root samples from an uprooted, declining grapefruit tree on sour orange rootstock in a residential property in Mission, Texas. Dry root rot symptoms displaying patchy, black discoloration and decaying roots (A & D); longitudinal sections showing reddish-purple to black discoloration limited to a section (B & C).

Fungal colonies resembling *F. solani* were subcultured on carnation leaf agar (CLA) (Fisher et al. 1982) culture plates, and purified using the single-spore technique. Isolates growing on CLA were examined microscopically and identified to species based on the most distinctive morphological characteristics according to Leslie and Summerell (2006). Isolates were also grown on potato dextrose agar (PDA) (Nelson et al. 1983) for the development of color.

Pathogenicity test

Nine isolates were recovered from the root tissue. Since all isolates growing on PDA were identical in morphology, a single isolate was selected randomly. This isolate was grown at 25 °C on CLA under two 40 W black light and two 40 W cool fluorescent tubes for 7 days with a 12 h photoperiod to induce sporulation (Burgess et al. 1988). Spores were collected by rinsing plates with sterile water and then filtering the water through 4 layers of sterile cheesecloth. The spore suspension, including macroconidia and microconidia, was adjusted to a concentration of 200,000 CFU/ml and used as inoculum. Eight months old sour orange rootstock seedlings grown in Metro-Mix professional growing mix (Sungro Horticulture, Agawam, MA) were uprooted and shaken to eliminate most of the growth medium. The tips of the rootlets were cut with sterile scissors, and thereafter dipped into 200 ml of the spore suspension for 60 s. Inoculated seedlings were re-planted in the same growth medium in cone-containers (cell diameter of 3.8 cm and a depth of 21 cm). Seedlings used as controls were treated similarly, but dipped into sterile water. Seedlings were maintained in a greenhouse. After 9 months, plants were uprooted for re-isolation of the pathogen as described above and evaluation of symptoms.

DNA extraction and PCR

Total DNA was isolated using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA) from both lesion tissue on the roots and directly from the fungal mycelium grown for 10 days on Difco™ PDA at 26 °C. A total of 8 fungal DNA extracts, including 1 from initial infected root tissue and 7 from fungal isolates obtained 9 months post-inoculation of sour orange rootstock plants, were submitted to PCR to obtain nucleotide sequence information. The PCR amplification was performed using either *F. solani* species-specific primers based on internal transcribed spacer region (ITS), ITS-Fu2f/ITS-Fu2r, or transcription elongation factor (TEF-1α)-based primers, TEF-Fs4f/TEF-Fs4r (Arif et al. 2012). The most commonly used primers for fungal identification based on nucleotide sequences, ITS3/ITS4 (White et al. 1990), were also used. The PCR amplification products were separated by electrophoresis on 1% agarose gels, stained with ethidium bromide, visualized under ultraviolet light, and photographed using Biospectrum imaging system (UVP, Upland, CA). Thin slices of agarose gel containing the amplicon DNA were cut, and the DNA sequenced at MCLAB (San Francisco, CA). The nucleotide sequences were analyzed for similarities via the National Center for Biotechnology Information's (NCBI) database, using the standard nucleotide Basic Local Alignment Search Tool blastn algorithm and deposited into GenBank. The fungal cultures are available at the TAMUK Citrus Center, Weslaco, Texas, and USDA-APHIS, Edinburg, Texas. The isolates will be deposited into the American Type Culture Collection.

Results

Fungal colony

Colonies on PDA developed abundant mycelium with light purple-brownish pigmentation (Fig. 2). On CLA, macroconidia were wide, straight, stout, mostly 5 septate, blunt and with a somewhat rounded, beaked apical cell and a poorly developed, foot-shaped basal cell; microconidia were abundant, oval, ellipsoid or reniform, 0- to 1-septate, produced in false heads from long monophialidic conidiogenous cells (Fig. 2). Based on the above characteristics, the fungal isolate obtained from the purple discolored vascular tissue was identified as *F. solani* (Leslie and Summerell 2006; Nelson et al. 1983).

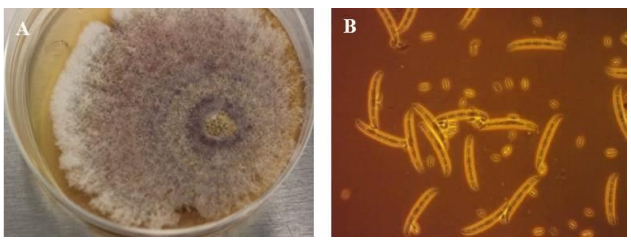

Fig. 2. (A) *Fusarium solani* growing on potato dextrose agar, showing light purple-brownish pigmentation; (B) microconidia and macroconidia on carnation leaf agar.

Pathogenicity assay

Symptom progression in inoculated sour orange seedlings was evident soon after inoculations. Seedlings showed yellowing and poor health (Fig. 3A). At 9 months, they were smaller in size than the non-inoculated seedlings (28 ± 7.5 cm vs 36 ± 4.8 cm). After 10 months, 2 plants out of 9 inoculated wilted and died. The root system of inoculated seedlings showed brownish discoloration, and numerous decaying roots (Fig. 3B). In contrast, control plants had asymptomatic root systems. Re-isolations made on Komada's medium from rootlets and from the tip, middle area, and at the soil level of the tap root of inoculated plants yielded the inoculated fungus *F. solani*, which was identified by morphology (Leslie and Summerell 2006; Nelson et al. 1983) and PCR.

Fig. 3. (A) Sour orange rootstock seedlings inoculated with *F. solani* (right) showing yellowing, poor health, and small in size compared to non-inoculated plants (left) after 2 months post-inoculation; (B) The root system of inoculated seedlings (left) showing brownish discoloration and decaying roots 9 months post-inoculation; uninoculated roots shown on the right.

PCR and DNA sequencing

The PCR amplification of DNA from fungal mycelium using *F. solani* species-specific primers, ITS-Fu2f/ITS-Fu2r and TEF-Fs4f/TEF-Fs4r, and universal fungal primers ITS3/ITS4, resulted in amplicons of sizes 375 bp (Fig. 4), 658 bp (Fig. 5), and 350 bp (Fig. 6), respectively.

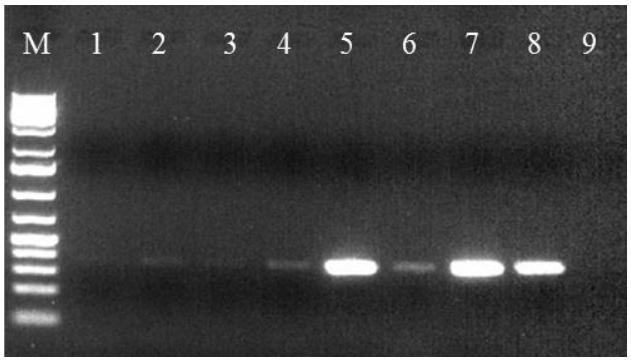

Fig. 4. Agarose gel showing PCR fragments produced by amplification of *Fusarium solani* isolate fungal DNA extracts using ITSFu2f/2r primers. Lanes 1-8: *F. solani* isolates from different plant parts 9 months post-inoculation, 1 = crown, 2 = stem middle, 3 = stem base, 4&7 = root base, 5 = roots middle, 6 = stem base, 8 = first isolate from the original infected root samples which was used in plant inoculations, 9 = non-template control, M = 1 kb plus Molecular marker (Fermentas).

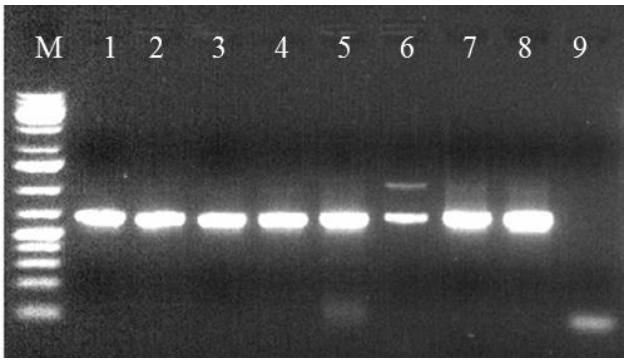

Fig. 5. Agarose gel showing PCR fragments produced by amplification of *Fusarium solani* isolate fungal DNA extracts using TEF4f/4r primers. Lanes 1-8: *F. solani* isolates from different plant parts 9 months post-inoculation, 1 = crown, 2 = stem middle, 3 = stem base, 4&7 = root base, 5 = roots middle, 6 = stem base, 8 = first isolate from the infected root samples which was used in plant inoculation, 9 = non-template control, M = 1 kb plus Molecular marker (Fermentas).

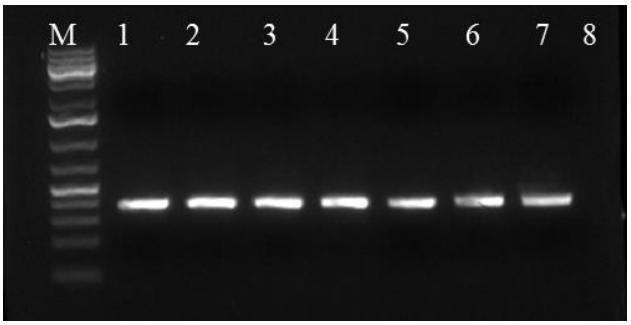

Fig. 6. Agarose gel showing PCR fragments produced by amplification of *Fusarium solani* isolate fungal DNA extracts using ITS3/ITS4 primers. Lanes 1 = crown, 2 = stem middle, 3 = stem base, 4&7 = root base, 5 = roots middle, 6 = stem base, 8 = non-template control, M = 1 kb plus Molecular marker (Fermentas).

Similarity searches for these amplicons using blastn showed 99% to 100% identity (Evalue 2e-150, 0.0, and 2e-154, respectively) to several *F. solani* nucleotide sequences. In comparison, the next closest blastn match for the nucleotide sequence obtained using TEF-Fs4f/TEF-Fs4r was to *F.* cf. *ensiforme* FRC S1847, a new species from the *F. solani* species complex (98% identity with E value 0.0) while all remaining matches were to *F. solani* sequences, thus providing diagnostic confirmation of the *F. solani* isolate. Nucleotide sequences obtained by ITS-Fu2f/ITS-Fu2r and ITS3/ITS4 primers showed the next closest blastn matches to be *Colletotrichum musae* (99% identity with E value 2e-150) and *F. oxysporum* (100% identity with E value 2e-154), respectively. When the *C. musae* sequence record was subjected to blastn search, it resulted in similarity to only 2 *C. musae* records from the same group that originally deposited these sequences from Sri Lanka, and the remaining matches were *F. solani* sequences. It is important to note that only the sequence obtained using universal ITS3/ITS4 primers showed identity to 1 entry of *F. oyxsporum* in GenBank while the other 2 sequences did not show any match to *F. oxysporum*. A total of 13 nucleotide sequences obtained

including 5 each for amplicons derived using TEF-Fs4f/TEF-Fs4r and ITS3/ITS4, and 3 for amplicons derived using ITS-Fu2f/ITS-Fu2r primers were deposited in NCBI's GenBank database (accession numbers: KR264881 through KR264893).

Discussion

The *F. solani*-infected grapefruit tree on sour orange rootstock growing in a dooryard in Mission, Texas, showed severe symptoms of wilt, tree decline, desiccation of leaves and leaf drop, discolored roots and root necrosis, purple discoloration of the wood, and dieback of the canopy. It was reported that no rootstock seedlings were resistant to *F. solani* infection (Nemec et al. 1980). In Florida, *F. solani* is a ubiquitous fungus in soil and around citrus tree roots (Nemec and Chaddock 1977; Nemec 1987) which can be pathogenic to citrus and has been associated with symptoms in trees with blight (Nemec et al. 1980). Trees that are stressed because of environmental factors, for example trees growing under poor drainage conditions, showed higher incidence of dry root rot compared to trees growing under no stress (Polizzi et al. 1992). In the 1980s, *F. solani* was associated with branch and trunk cankers on citrus trees weakened by freeze in Florida (Nemec 1987). Moreover, Graham et al. (1985) showed that *F. solani* only infected citrus trees that were weakened and had reduced vigor and root growth. They also concluded that *F. solani* appeared to be a primary colonizer of citrus tree roots with depleted starch reserves in the tree due to stress conditions. While studying the possible role of *F. solani* infection of citrus roots in blight etiology, Nemec et al. (1980) observed *F. solani* induced root rot and increased vessel plugging in roots and above ground parts in blighted trees.

The inoculated sour orange seedlings showed root rot 9 months after inoculation with an *F. solani* conidial suspension. The seedlings initially showed wilting after a single inoculation, but later recovered from it and grew normally. However, the inoculated seedlings showed slightly reduced growth compared to uninoculated control plants. Nine months after inoculation, the infection moved to above-ground plant parts and we successfully isolated it from stem pieces. We did not observe necrosis or purple discoloration in the stem. Studies in California and Italy suggested that citranges were more susceptible to dry root rot compared to sour orange (Menge et al. 1981; Polizzi et al. 1992). The isolate in this study was pathogenic on sour orange rootstock, which is the predominant rootstock used in south Texas citrus production and the basis of our inoculation studies.

Screening of a large number of citrus seedlings showed that resistance to *F. solani* is uncommon in citrus (Krueger and Bender 2015). Thus, there is a need to search for sources of resistance. Poor drainage, excessive irrigation, and poor aeration in the rhizosphere enhance the probability of *F. solani* infecting citrus trees. Citrus in Texas are often grown in heavy, sandy-clay-loam soils and are, traditionally, flood irrigated. This over watering combined with poorly draining soil provides an environment conducive to *F. solani* infection of citrus. The root rot and damage to the citrus root systems due to *F. solani* was significantly higher in trees co-infected with *Phytophthora* spp. Presence of *Phytophthora* predisposes citrus to *F. solani* infection by providing an opportunity for opportunistic colonization of the necrotic tissue (Dandurand and Menge 1994). Citrus trees affected by *Phytophthora* infections are common in Texas citrus orchards (Kunta et al. 2007). At present, no data are available on the amount of damage in Texas citrus caused by *F. solani* in association with *Phytophthora* spp. Therefore, it is important to survey the groves in Texas to estimate possible damage to citrus roots and the potential reductions in yield due to *F. solani* and *Phytophthora* interactions. Moreover, effective management of *Phytophthora* is presumably essential for management of dry root rot (Adesemoye et al. 2011).

References

Adesemoye A, Eskalen A, Faber B, Bender G, O'Connell N, Kallsen C, Shea T. 2011. Current knowledge on Fusarium dry rot of citrus. Citrograph. 2(7):29-33.

Arif M, Chawla S, Zaidi MW, Rayar JK, Variar M, Singh US. 2012. Development of specific primers for genus *Fusarium* and *F. solani* using rDNA sub-unit and transcription elongation factor (TEF-1α) gene. African J Biotech. 11:444-447.

Broadbent P. 2000. Dry root rot or sudden death. In: Timmer LW, Garnsey SM, Graham JH, editors. Compendium of citrus diseases. 2nd ed. St Paul (MN): APS Press. p. 71.

Burgess LW, Lidell CM, Summerell BA. 1988. Laboratory manual for *Fusarium* research. Sydney (Australia): The University of Sydney.

Dandurand LM, Menge JA. 1994. Influence of *Fusarium solani* on chemotaxis of zoospores of *Phytophthora parasitica* and *Phytophthora citrophthora* and on distribution of 14C in citrus tissues and root exudate. Soil Biol and Biochem. 26:75-79.

Fisher NL, Burgess LW, Toussoun TA, Nelson PE. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. Phytopathology. 72:151–153.

Graham JH, Brlansky RH, Timmer LW, Lee RF, Marais, LJ, Bender GS. 1985. Comparison of Citrus tree declines with necrosis of major roots and their association with *Fusarium solani*. Plant Dis. 69:1055-1058.

Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev Plant Prot Res. 8:114-175.

Krueger RR, Bender GS. 2015. Screening a core collection of citrus genetic resources for resistance to *Fusarium solani*. Acta Hort. 1065:155-163.

Kunta M, Hongqin M, Skaria M. 2007. Molecular distinction of *Phytophthora* isolates in the Lower Rio Grande Valley of Texas. Subtrop Plant Sci. 59:1-5.

Leslie JF, Summerell BA. 2006. The *Fusarium* laboratory manual. Oxford (England): Blackwell Publishing.

Menge JA, Johnson ELV, Sibert D, Burns RM. 1981. Dry root rot of citrus – A disease complex [abstract]. Phytopathology. 71:242.

Nelson PE, Toussoun TA, Marasas WFO. 1983. *Fusarium* species: An illustrated manual for identification. University Park (PA): Pennsylvania State University Press.

Nemec S. 1987. *Fusarium solani* association with branch and trunk cankers on citrus weakened by cold weather in Florida. Mycopathologia. 97:143-150.

Nemec S, Baker R, Burnett H. 1980. Pathogenicity of *Fusarium solani* to citrus roots and its possible role in blight etiology. Proc Florida State Hort Soc. 93:36-41.

Nemec S, Chaddock A. 1977. *Fusarium* populations in Florida citrus soils [abstract]. Phytopathology. 4:116-117.

Okamura JK, Davis RM. 1987. Latent and saprophytic fungal infections of grapefruit in south Texas. Texas J Agric Nat Resources. 1:19-20.

Polizzi G, Magnano di San Lio G, Catara A. 1992. “Dry root rot” of citranges in Italy. Proc Int Soc Citriculture. 2:890-893.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego (CA): Academic Press. p. 315-322.