Seedborne *Fusarium neocosmosporiellum* causing Foot Rot-Induced Wilting in Peanut Identified in Texas.

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Abstract

A test of a batch of peanut (*Arachis hypogaea* L.) seed variety of the Runner-type in 2021 in Texas revealed seed-associated *Fusarium neocosmosporiellum*. The identity of the *Fusarium* isolate was confirmed based on its morphological characteristics as well as the internal transcribed spacer (ITS) and translation elongation factor 1-alpha (TEF) sequences, respectively. The inoculum potential of the *Fusarium* isolate was also evaluated on plants derived from *F. neocosmosporiellum*-free peanut seeds using the method of Koch's postulate. The results of pathogenicity tests determined the fungus to be pathogenic on peanut plants, resulting in foot rot-induced plant wilting and decline.

In peanuts, seed testing prior to planting has been recommended to growers as a preventive measure against the new disease Bacterial Early Decline. This disease, caused by two pathogens belonging to the genera *Ralstonia* sp. and *Pantoea* sp., caused a devastating outbreak in 2020 in the Panhandle of Texas (Obasa and Haynes 2022). Consequently, during the testing of a batch of grower's seeds, variety GA-09B (Runner-type), in 2021 at the Texas High Plains Plant Disease Diagnostic Laboratory, a fungus with morphological characteristics identical to those of *F. neocosmosporiellum* (syn. *Neocosmospora vasinfecta*) (Geiser et al. 2013) was isolated from some of the peanut seeds on Luria Bertani (LB) agar plates (Figure 1a). The fungus colony was light-buff in color with a light purple hue around the center of the colony and produced abundant orange-red fruiting bodies. Microscopy examinations of the fruiting bodies produced on quarter-strength potato dextrose agar (1/4 PDA) maintained at 25°C in the dark, after colony purification by hyphal-tipping, revealed them as asci-containing perithecia (Figure 1b) and consistent with *F.*

neocosmosporiellum (Dau et al. 2010). The diameter of the perithecium averaged ~750 µm (range ~695-925 µm) (Figure 1b). Asci contained ovoid-shaped and single-celled pale-yellow ascospore with average length of ~24 µm (range ~20-39 µm) (Figure 1c). Further identification of the fungus was inferred using DNA sequences. The genomic DNA of the fungus was isolated using the Plant/Fungi Genomic DNA isolation kit by Norgen Biotek Corp. (Ontario, Canada), and according to the manufacturer's instructions. The internal transcribed spacer (ITS) gene sequence fragment was amplified by polymerase chain reaction (PCR) using the primer pair ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) (White et al. 1990) at a final concentration of 0.5 µM and 1× final concentration of Bio-rad 2× HF Master-Mix (BIO-RAD, Hercules, CA) in a 25 µl total reaction volume. The PCR amplification cycle consisted of an initial denaturing step at 98°C for 3 min, 35 cycles of three steps consisting of 98°C for 10 sec, 55°C for 30 sec, 72°C for 30 sec, and a final step of 72°C for 5 min. The resulting PCR product, ~500 bp amplicon, was verified by gel electrophoresis, purified, and sequenced. The obtained sequence was used to identify closely-related species by BLASTN (Altschul et al. 1990). Finally, a phylogenetic tree was constructed based on the translation elongation factor 1-alpha gene sequence (TEF) of the fungal isolate and those of closely relates species identified by BLASTN, as well as representatives of other *Fusarium* species, using the Tamura-Nei genetic distance model (Tamura and Nei 1993). No outgroup was included in the phylogenetic analysis. The default settings of the Geneious Tree Builder application in Geneious Prime (Kearse et al. 2012) was used for building the phylogenetic tree. The TEF gene sequence was obtained from the do-novo assembled and annotated draft genome derived from 2 × 150 bp paired-end genomic DNA sequencing reads generated on the Illumina NovaSeq PE150 sequencing platform. Library for sequencing was generated with the NEBNext DNA Library Prep Kit (New England Biolabs, Ipswich, MA). The generated raw reads were processed to remove adapters and primers and the processed reads subsequently assembled using the default settings of the Geneious SPAdes DNA seq de novo assembler (Biomatters, Inc., San Diego, CA; Bankevich et al. 2012, Gurevich et al. 2013). The draft genome of the fungal isolate will be presented elsewhere.

Two independent pathogenicity assays to evaluate the inoculum potential of the *Fusarium* isolate was conducted using healthy peanut seeds of the Runner-type variety under greenhouse conditions as previously described by Obasa and Haynes (2022) with slight modifications.

Briefly, surface disinfected (0.5% NaOCl for about 2 min) and air-dried healthy peanut seeds were seeded at a rate of four seeds per pot in sterile potting media (Berger BM1 Nutrient Retention General Purpose Media; Hummert International, Topeka, KS) contained in 1.87 L plastic pots. Three replicate pots per treatment were used. The pots were lightly watered daily and maintained at 28°C for 17 days. At 14 days post-germination, whole seedling plants, with their roots, were removed from their respective potting media, their roots rinsed in sterile-distilled water and briefly air-dried, and the roots subsequently immersed by dipping in the fungus' inoculum for an hour. The fungal inoculum was comprised of 8-day-old mycelia grown in quarter strength potato dextrose broth at 28°C and 150 rpm on a shaker. The mycelia were subsequently macerated for approximately 10 secs in a Conair™ Waring™ Laboratory Blender using the lowest speed (18,000 rpm). Following the inoculation period in the fungal inoculum, the inoculated plants were replanted in sterile potting media and maintained under the same pre-inoculation greenhouse conditions. Non-inoculated plants dipped in sterile-distilled water for one hour served as controls.

The BLASTN search of the sequenced 483 bp ITS fragment of the fungal isolate (GenBank accession: ON364133) returned a hundred percent identity match, for the complete query sequence, to corresponding sequences of isolates belonging to *F. neocosmosporiellum* and *Neocosmospora vasinfecta*. Similar BLASTN search of a 650 bp partial sequence of the isolate's translation elongation factor 1-alpha (TEF) gene returned a hundred percent identity match with corresponding *F. neocosmosporiellum* isolates in the database. Pairwise alignment searches of the 483 bp ITS and 650 bp TEF sequence fragments in the CBS-KNAW Fungal Biodiversity Center's *Fusarium* MLST website (https://fusarium.mycobank.org) returned a 99.59% and 98.62% top identity match respectively to corresponding *F. neocosmosporiellum* reference isolates. The result of phylogenetic analysis based on a 650 bp sequence of the fungus' TEF gene (GenBank accession: ON456283) similarly indicated that the peanut *Fusarium* isolate is closely related to *F. neocosmosporiellum* (Figure 2). The isolate is heretofore designated as *F. neocosmosporiellum* isolate F303-D. Two independent greenhouse pathogenicity assays conducted to evaluate the inoculum potential of the peanut seedborne *Fusarium* isolate resulted in foot rot-induced plant wilt in all inoculated plants (Figure 1b, d, f). Observed symptoms of the disease included development of dark-brown to black necrotic lesions in the lower stem and crown regions, and dark-brown root discoloration and necrosis. The non-inoculated control

plants showed no signs of wilt nor foot rot (Figure 1c, e, g). There are very few documented cases of *F. neocosmosporiellum* infecting peanut in the U.S., and earlier documented cases of *F. neocosmosporiellum* in the U.S. involved isolations from peanut pods (Texas), from soils (Florida and Georgia), and from cowpea (Alabama and South Carolina) (Ramirez et al. 2022). This finding therefore represents the first reported case of peanut seed-associated *F. neocosmosporiellum* in the U.S. and further underscores the importance of peanut seed testing, especially by peanut farmers, prior to planting. Such a practice can help identify potential disease risk factor(s) in seed batches and in turn help to mitigate the potential economic losses from subsequent infection of field crops by seedborne peanut pathogens.

Acknowledgments

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Literature Cited

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. Journal of Molecular Biology 215(3):403-10 (doi: 10.1016/S0022-2836(05)80360-2. PMID: 2231712).

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., and Pevzner, P. A. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of computational biology 19(5): 455-77 (doi: 10.1089/cmb.2012.0021. Epub 2012 Apr 16. PMID: 22506599; PMCID: PMC3342519).

Dau, V. T., Pham, L. T., Luong, T. M., Huynh, L. M. T., Tran, N. T., Ho, T. D., Hoang, H. M. T., Phan, H. T., and Burgess, L. W. 2010. First report of *Neocosmospora vasinfecta* associated with the root rot complex of peanuts in Vietnam. Australasian Plant Disease Notes, 5: 79–81.

Geiser, D. M., Aoki, T., Bacon, C. W., Baker, S. E., Bhattacharyya, M. K., Brandt, M. E., et al. 2013. One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. *Phytopathology* 103, 400–408. doi: 10.1094/PHYTO-07-12-0150-LE.

Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics (Oxford, England) 29(8):1072-1075.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., and Drummond, A. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12), 1647-1649.

Obasa, K. and Haynes, L. 2022. Two bacterial pathogens of peanut, causing early seedling decline disease, identified in the Texas Panhandle. Plant Disease 106: 648-653. https://doi.org/10.1094/PDIS-07-21-1555-RE.

Ramirez, J., Watson, K., Thiers, B., and McMillin, L. 2022. The New York Botanical Garden Herbarium (NY). Version 1.45. The New York Botanical Garden. Occurrence dataset https://doi.org/10.15468/6e8nje accessed via GBIF.org on 2022-04-22. https://www.gbif.org/occurrence/1928265896.

Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10(3): 512–526.

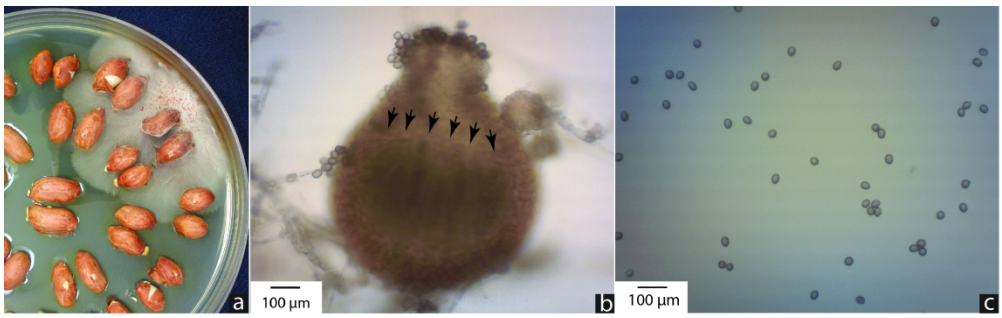
White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand, D. H., Snisky, J. J., White, T. J., eds. PCR protocols: a guide to methods and applications. San Diego: Academic Press. p 315–322.

Figure Legends

Figure 1. Peanut seed-derived *Fusarium neocosmosporiellum* (a), and microscopy image (200× magnification) of (b) asci-containing (black arrow) perithecium with some ascospores, and (c) pale-yellow colored ascospores.

Figure 2. Pathogenicity assays evaluating the inoculum potential of *Fusarium neocosmosporiellum* isolate F303-D on peanut plants under greenhouse conditions showing development of foot rot-induced wilt symptoms on inoculated plants at 6 (b) and 11 (d) days post-inoculation (dpi), also with visible foot rot (f) at 11 dpi on the stems of inoculated plants. The non-inoculated control plants developed neither wilt nor foot rot (a, c, and e).

Figure 3. Phylogeny of *Fusarium neocosmosporiellum* isolate F303-D with forty closely related *Fusarium* species using the translation elongation factor 1-alpha (TEF) gene sequence showing their relatedness. Bar indicates branch length, estimated using the Tamura-Nei genetic distance model.



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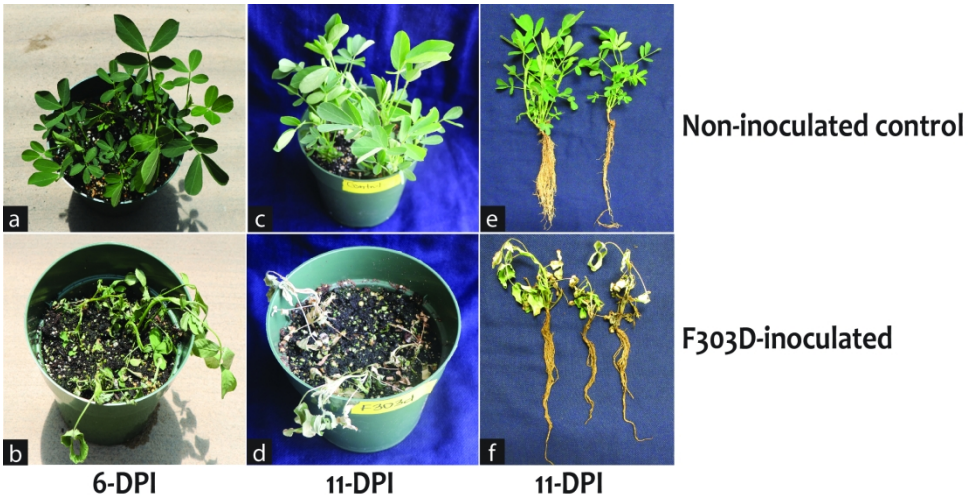

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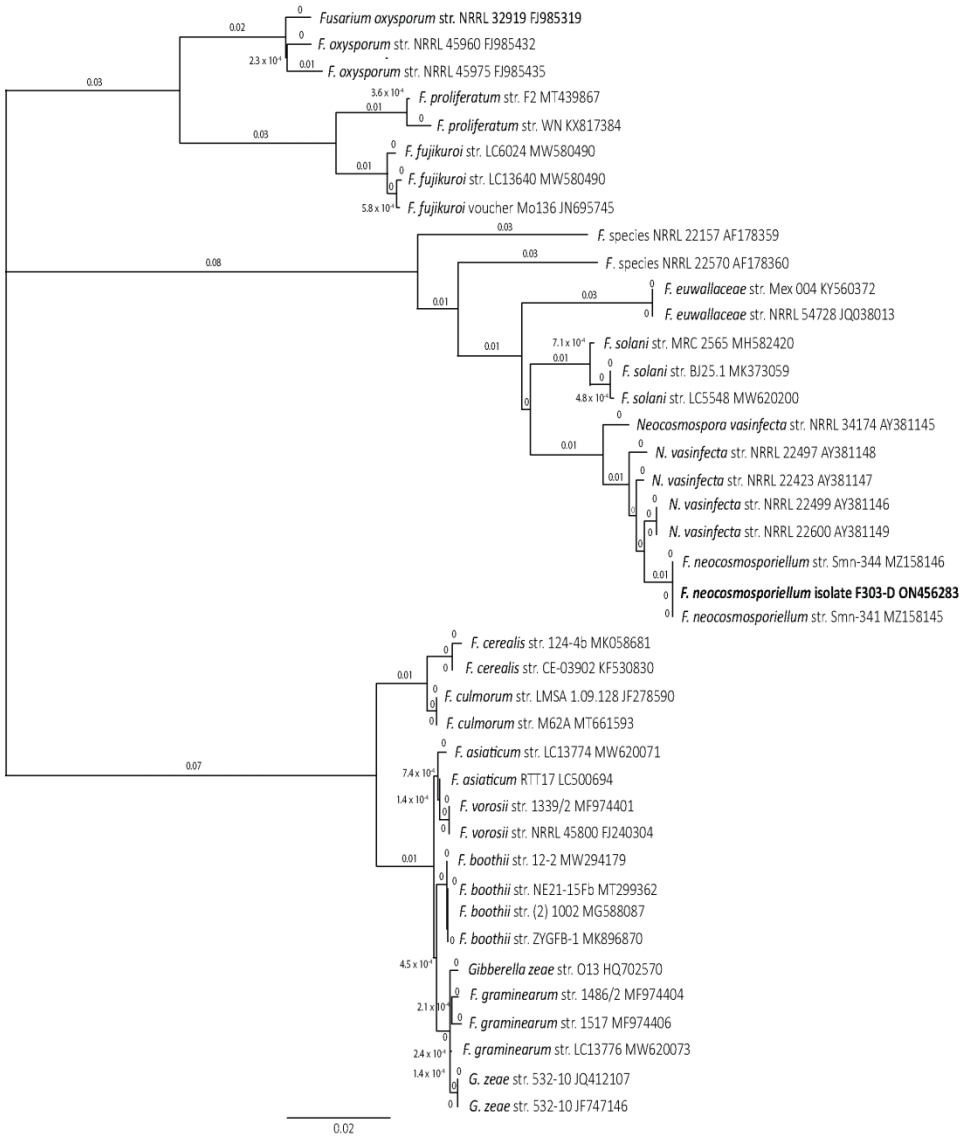

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