First Report of Pathogenicity of *Fusarium sporotrichioides* and *Fusarium acuminatum* on Sunflowers in the United States

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A sunflower field (*Helianthus annuus* L. cv. 'Pioneer 63M82') with uneven maturation was observed in Todd County, MN, in September 2009 (2). Thirty-seven percent of the plants were wilted and approximately 12% of the plants had lesions consistent with charcoal rot [*Macrophomina phaseolina* (Tassi) Goid.] despite a sub-optimal environment for disease development (2,3). Stem sections of the basal portion of infected plants were harvested and dissected. In addition to spherical microsclerotia consistent with *M. phaseolina* (3), a pink discoloration of the pith was observed (Figs. 1 and 2).

![Fig. 1. Transverse sections of sunflower stalks collected from field near Aldrich, MN, exhibiting a range of signs and symptoms caused by *Macrophomina phaseolina* (note microsclerotia) and *Fusarium* spp. (note pink discoloration).](image1)

![Fig. 2. Close-up of transverse sections of sunflower stalks collected near Aldrich, MN, exhibiting pink discoloration caused by *Fusarium* spp., and microsclerotia of *M. phaseolina* in pith.](image2)

Small pieces of pink pith were surface-disinfected in 0.5% sodium hypochlorite and 70% ethanol for 60 s each, rinsed in sterile distilled H₂O, transferred to potato dextrose agar (PDA) in petri dishes and incubated for 7 days at 22° to 25°C under fluorescent lights with a 12-h photoperiod. Macroconidia from aerial hypha were harvested and streaked on 1.5% water agar and after 3 days, individual colonies were transferred to PDA to record colony morphology, density, and extent of mycelia growth. Cultures from single-spore isolates formed characteristic pale pink to purple colonies, floccose mycelium, and slow growth. In addition to colony morphology, *Fusarium* spp. were identified by morphological characteristics of macroconidia as *Fusarium oxysporum* Schlechtend: Fr. (three septate, 23-54 × 3-4.5 µm); *F. sporotrichioides* Ellis & Everh. (primarily three septate, 39-51 × 4-5 µm); and *F. acuminatum* Sherb (primarily three septate, long tapering apical cell, 30-55 × 3-4 µm) (4). DNA was extracted from lyophilized mycelium of the latter two *Fusarium* spp. using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) and its translation elongation factor 1-alpha (TEF1-α) gene region was amplified using...
ef1 and ef2 primers (6). PCR amplicons of ~700 bp were directly sequenced in both directions using the same primers, and a BLASTN search against the NCBI nucleotide database was performed using the consensus sequence generated by alignment of the forward and reverse sequences for this region. BLASTN search results confirmed 99% identity with \textit{F. sporotrichioides} (closest match was accession no. EU744849) and 100% identity with \textit{F. acuminatum} (closest match was accession no. FJ154737).

Koch’s postulates were performed in a greenhouse with air temperatures ranging from 20° to 25°C and a 14-h photoperiod. For each pathogen, ten 3-week-old sunflower plants (cv. ‘Pioneer 63M82’) at the six to eight leaf growth stage were inoculated by securing mycelia agar plugs of 7-day-old cultures (PDA) with parafilm on stems wounded with sterile pipette tips using a method modified from Zazzerini and Tosi 1987 (8). The experiment was repeated twice. Necrosis was observed 10-days after inoculation with each pathogen. Plants inoculated with \textit{F. sporotrichioides} exhibited necrotic girdling lesions on 100% of plants at 14-day post-inoculation, and wilting occurred on 80% of the plants by 21-days post inoculation (Fig. 3). Girdling necrotic lesions developed on plants inoculated with \textit{F. acuminatum} at 14-days post inoculation, but wilting was not observed by 21-days when experiments were terminated. No plants inoculated with sterile agar plugs had symptoms. Both \textit{Fusarium} spp. were re-isolated from necrotic tissue fragments within 3-cm of the inoculation point of five randomly-selected inoculated-plants per pathogen, fulfilling Koch’s postulates. Association of \textit{M. phaseolina} and an unidentified \textit{Fusarium} spp. was previously reported in the United States (7). Despite their co-occurrence, Orellana (7) found that the \textit{Fusarium} (then unidentified spp.) caused wilt and was more aggressive than \textit{M. phaseolina}. In this brief, the specific \textit{Fusarium} species causing disease on sunflowers in Minnesota were identified and pathogenicity was demonstrated but the relationship among them and \textit{M. phaseolina} was not investigated. The occurrence of \textit{M. phaseolina} and pathogenic \textit{Fusarium} spp. may have implications for accurate diagnoses of the primary pathogen of diseased sunflowers. Sunflower plants infected by \textit{M. phaseolina} exhibit visually recognizable symptoms and are easily identified by the presence of microsclerotia, but the presence of pink tissue (caused by \textit{Fusarium} spp.) in stalks should not be assumed to be a secondary infection. In a disease survey of sunflower fields conducted between 1999 and 2001 in the Krasnodar region in Russia, sunflowers with wilt symptoms were collected and \textit{F. oxysporum}, \textit{F. solani}, and \textit{F. sporotrichioides} were the most common species recovered (1). When the effect of \textit{F. oxysporum} and \textit{F. sporotrichioides} isolates on sunflower emergence were compared, \textit{F. sporotrichioides} reduced emergence more than \textit{F. oxysporum} (1). \textit{F. sporotrichioides} was commonly found on wheat-bioassays in North Dakota (5), the leading producer of sunflowers in the United States and a border state to Minnesota. Despite pathogen ubiquity in the region, current and future implications of \textit{Fusarium} spp. on disease incidence, severity and yield loss are unclear. To our knowledge, this is the first report of \textit{F. sporotrichioides} and \textit{F. acuminatum} causing disease on \textit{Helianthus annuus} in the United States.
Literature Cited


