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Anthracnose disease of switchgrass caused by the novel fungal species *Colletotrichum navitas*

*Jo Anne CROUCH**, *Lisa A. BEIRN*, *Laura M. CORTESE*, *Stacy A. BONOS*, *Bruce B. CLARKE*

Rutgers University, Department of Plant Biology and Pathology, New Brunswick, NJ 08901, USA

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ABSTRACT

In recent years perennial grasses such as the native tallgrass prairie plant *Panicum virgatum* (switchgrass) have taken on a new role in the North American landscape as a plant-based source of renewable energy. Because switchgrass is a native plant, it has been suggested that disease problems will be minimal, but little research in this area has been conducted. Recently, outbreaks of switchgrass anthracnose disease have been reported from the northeastern United States. Incidences of switchgrass anthracnose are known in North America since 1886 through herbarium specimens and disease reports, but the causal agent of this disease has never been experimentally determined or taxonomically evaluated. In the present work, we evaluate the causal agent of switchgrass anthracnose, a new species we describe as *Colletotrichum navitas* (*navitas* = Latin for energy). Multilocus molecular phylogenetics and morphological characters show *C. navitas* is a novel species in the falcate-spored graminicolous group of the genus *Colletotrichum*; it is most closely related to the corn anthracnose pathogen *Colletotrichum graminicola*. We present a formal description and illustrations for *C. navitas* and provide experimental confirmation that this organism is responsible for switchgrass anthracnose disease.

Introduction

Perennial grasses such as switchgrass (*Panicum virgatum*) are currently the focus of intensive study as potential high-energy alternative feedstocks for the production of biofuels in the United States (for review, see Keshwani & Cheng 2009). As the biofuel industry expands in the U.S. and more switchgrass is grown in monoculture, diseases and insect pests may pose significant problems for switchgrass production if the organisms capable of establishing pathogenic relationships with this grass are not assessed during the breeding process. Because switchgrass is a widely dispersed North American native plant, it has been suggested that long-term exposure to native fungi, bacteria and other microbes, in combination with pathogen screening and the use of genetically diverse cultivars will be sufficient to limit disease (Mitchell *et al.* 2008). Unfortunately, such predictions about switchgrass disease dynamics are highly speculative. Resistance and host diversity are not always sufficient in combating diseases that impact perennial grass crops, particularly in cases where the pathogen is relatively immobile and capable of inhabiting plant residues, soil, or the rhizosphere (reviewed in Cox *et al.* 2005).

Eighty-one species of fungi have been reported to cause disease in switchgrass in the United States (summarized in

* Corresponding author. Present address: Cereal Disease Laboratory, U.S. Department of Agriculture, Agricultural Research Service, 1551 Lindig Street, St. Paul, MN 55108, USA.
E-mail addresses: joanne.crouch@ars.usda.gov, crouch@aesop.rutgers.edu
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Farr & Rossman 2009); however, almost no research has been performed in this area, presumably because there has been little economic impact. Even for those few switchgrass fungal pathogens that have been described in the primary literature, the majority of the publications are reports of incidence (Carris et al. 2008; Gravert et al. 2000; Gravert & Munkvold 2002; Gustafson et al. 2003; Krupinsky et al. 2004; Zale et al. 2008; Zeiders 1984). One fungal disease that may prove problematic for switchgrass cultivation in the U.S. is anthracnose, with incidences of this disease recently reported from the states of Iowa, Tennessee, North Carolina, Pennsylvania, New York and New Jersey (Gravert & Munkvold 2002; Li et al. 2009; Sanderson 2008; Bergstrom & Waxman personal communication; this study). Fungi in the genus *Colletotrichum* are thought to cause switchgrass anthracnose, although Koch's postulates have never been tested to confirm the causal agent. Historically, the disease is largely documented through reports in technical bulletins and herbarium specimens (Anonymous 1960; Greene 1944; Mankin 1969; Ray 1954; Rogerson 1958; Sprague 1950). The earliest known record of switchgrass anthracnose in North America is from an herbarium specimen originally collected from *P. virgatum* in Louisiana in 1886 (currently curated by the U.S. National Fungus Collection, BPI), labeled *Colletotrichum lineola*, the name of the type species for the genus (currently known as *Colletotrichum dematium*; Arx 1957). In the first and only known taxonomic treatment to incorporate the switchgrass anthracnose fungus, Wilson included this early sample of *Colletotrichum* from *Panicum* along with samples from numerous other grasses within his circumscription of the wide host-range species *Colletotrichum graminicola* (Wilson 1914). Subsequent taxonomic, phylogenetic and experimental research demonstrated that *C. graminicola* is limited to establishing host associations with corn (*Zea mays*), leaving the identity of *Colletotrichum* associated with grasses other than corn undetermined (Sutton 1980, 1992; reviewed in Crouch & Beirn in press; Hyde et al. in press), including that of the switchgrass anthracnose fungus. Later, molecular phylogenetic study was used to diagnose and describe twelve additional species of morphologically cryptic *Colletotrichum* associated with grass hosts that were formerly part of *C. graminicola sensu lato* Wilson (Crouch et al. 2006, 2009a, b). But the *Colletotrichum* presumed to be responsible for switchgrass anthracnose disease remains unstudied and taxonomically undefined, and, for lack of any reasonable alternative, continues to be inaccurately referred to as *C. graminicola* (*e.g.* Gravert & Munkvold 2002; Li et al. 2009; Sanderson 2008). Thus, the first step in the study of switchgrass anthracnose is to determine the true identity of the fungus responsible causing the disease.

In this study, we use multilocus molecular phylogenetics, morphology and experimental inoculations to establish the identity of the switchgrass anthracnose pathogen and its relationship with other members of the grass-associated *Colletotrichum* group. Consistent with the high level of correspondence between host association and species boundaries that has been previously reported in the warm-season grass-associated *Colletotrichum* (Crouch et al. 2009a), our data show that anthracnose disease of switchgrass is caused by a novel species of *Colletotrichum*, which we describe as *Colletotrichum navitas* sp. nov.

Methods

Fungal isolates and morphological characterizations

Leaf and stem tissue was collected from *Panicum virgatum* cultivars or selections 'Cave-in-Rock', 'NSU 2001–01' and 'Brooklyn' (USDA Plant Materials Center, Cape May, NJ, USA) symptomatic for anthracnose disease on 26 Sept. 2008 at the Rutgers University Adelphia Research Center in Freehold, NJ, USA. These plants were originally established in 2005 for the purpose of cultivar evaluation trials. Fungi were established in pure culture by sectioning symptomatic leaf and stem tissue into small pieces (approximately 1–2 cm) and placing on the surface of potato dextrose agar (PDA; Fisher Scientific, Hampton, NH, USA) supplemented with 40 $\mu g\,L^{-1}$ each of penicillin, ampicillin, gentomycin, and streptomycin, followed by transfers to unamended PDA. Morphological evaluations of hyphae, setae, conidia, hyphal appressoria shape and size ($n = 50$, repeated twice) were performed as previously described (Crouch et al. 2009b).

Experimental tests of pathogenicity

Two distinct fungal colonies were isolated from the diseased plant tissue: one morphologically consistent with members of the genus *Colletotrichum*, and a second consistent with the genus *Nigrospora*. To determine which of these fungi was the causal agent of the observed disease symptoms, 8-wk old seedlings of *Panicum virgatum* (selection 'Brooklyn') were inoculated separately under controlled environmental conditions. Three replicate plants per treatment were sprayed with a 20 ml spore solution (5×10^4 conidia ml^{-1} in 0.1× potato dextrose broth (PDB; Fisher Scientific, Hampton, NH, USA); control plants were sprayed with 20-ml of sterile 0.1× PDB. Inoculated plants were placed in unsealed 2 ml 24″ × 36″ transluscent plastic autoclave bags (Fisher Scientific, Hampton, NH, USA) and placed in a growth chamber maintained at 30 °C, 60 % RH, 16-h d/8-h night. The experiment was repeated on 12-wk old *P. virgatum* seedlings.

Molecular analyses

Genomic DNA was isolated using a standard phenol:chloroform protocol as previously described (Crouch et al. 2005). For *Colletotrichum* isolates from switchgrass, four genes from three loci were amplified and sequenced as previously described: the internal transcribed spacer region (ITS; White et al. 1990), and portions of *Sod2* (Crouch et al. 2006), *Mat1* and *Apn2* (Crouch et al. 2009a). For *Nigrospora* isolates, the ITS region was amplified and sequenced. DNA sequence reactions were performed using ABI Prism BigDye 3.1 chemistry at 1/12 the manufacturer's recommended enzymatic volume (Applied Biosystems Inc., Foster City, CA, USA). Cycle sequencing reactions were cycled 99 times on an ABI Dual 96-Well GeneAmp PCR System 9700 (Applied Biosystems Inc., Foster City, CA, USA), primed from either (1) the PCR amplification primers for direct sequencing of DNA used in phylogenetic analyses, or (2) T7 and SP6 primers for plasmid insert