Research

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Late Growth Stages of Johnsongrass Can Act as an Alternate Host of *Colletotrichum sublineola*

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Abstract

Twenty-six johnsongrass cultivars, collected from seven U.S. states, were spray inoculated with spores of an isolate of *Colletotrichum sublineola* derived from sorghum. Inoculations were made when plants reached growth stages of 2 (four to five leaves), 3 (seven to eight leaves), and 6 (post panicle emergence). At the earlier stages the johnsongrass cultivars showed hypersensitive responses and small lesions; however, acervulus formation was not observed. When late growth stage johnsongrass plants were spray inoculated, acervulus formation was

confirmed in 19 cultivars as early as 5 days postinoculation. Results obtained confirm that johnsongrass can act as an alternate host of *C. sublineola* under favorable conditions, and growth stages of johnsongrass may be an important factor for cross infection of *C. sublineola* between johnsongrass and sorghum.

Keywords: weed science, sorghum, growth stage, alternate host, *Sorghum halepense*, anthracnose

Sorghum (*Sorghum bicolor* [L.] Moench) is the fifth most important cereal grown worldwide (Doggett 1988). Johnsongrass (*Sorghum halepense* [L.] Pers.), a wild relative of sorghum, is considered a noxious weed (Ohadi et al. 2018). The genomic relationship between cultivated sorghum (2n = 20) and johnsongrass (2n = 40) has been a subject of extensive studies (Arriola and Ellstrand 1996; Hadley 1958; Hoang-Tang and Liang 1988). *Colletotrichum sublineola* (Henn.) is an important disease of cultivated sorghum worldwide (Xavier et al. 2018). Although classified as *Colletotrichum graminicola* (Ces.) originally, it is now recognized that *C. sublineola* is a separate species that causes anthracnose leaf and stalk disease of sorghum, whereas *C. graminicola* is host specific for maize (*Zea mays* L. ssp. *mays*) (Buiate et al. 2017). Due to the genetic similarity of sorghum and johnsongrass, it was hypothesized that johnsongrass is a reservoir for *C. sublineola*, and cross-infection of *C. sublineola* was tested between sorghum and johnsongrass (Ahn et al. 2019). Recently, it was reported that 23 isolates of *C. sublineola* from cultivated sorghum were all pathogenic to at least one of 13 heritage inbred lines of *S. bicolor*; however, only four of 10 isolates from *S. halepense* caused disease on any of the same cultivars (Xavier et al. 2018). Furthermore, that study found that differences in DNA-based markers, including random amplification of

polymorphic DNA, and Southern blots using a probe with a transposon-like signature separated *C. sublineola* isolates from the two hosts into separate groups with little overlap. In the reverse direction, following spray inoculation of 26 johnsongrass cultivars with spores of *C. sublineola* isolate FSP53 from sorghum, the johnsongrass showed signs of induced defense response; however, acervulus formation was detected only on one leaf of one cultivar (SH1116) (Ahn et al. 2018). Furthermore, Ahn et al. (2018) conducted an excised leaf assay on the same cultivars with isolates FSP2, FSP35, and FSP53. Among the three isolates tested, FSP35 caused the most severe damage, with frequent acervulus formation on excised johnsongrass leaves (Ahn et al. 2018). Although confirming that cross-infection could lead to successful reproduction of the pathogen, based on greenhouse pathogenicity tests, the incidence was very rare on johnsongrass. Typically, growth stage 3 (eight leaf stage) plants are used for greenhouse pathogenicity tests for sorghum anthracnose, and our previous study also followed the procedure described by Prom et al. (2009). In a recent study, strong positive correlations between anthracnose severity and sorghum crop stage were found (Tsedaley et al. 2016). The experiments described here were designed to test two hypotheses: (i) *S. halepense* can act as an alternate host for an isolate of *C. sublineola* from sorghum, and (ii) growth stage of *S. halepense* at the time of inoculation affects the host–pathogen interaction. In order to test these hypotheses, 26 cultivars of johnsongrass collected from various geographical locations of the United States were spray inoculated with *C. sublineola* (isolate FSP35) at the four-leaf stage, at the eight-leaf stage, and after full panicle exertion. Based on an excised leaf assay, johnsongrass leaves were most susceptible to FSP35 isolate among the three tested isolates (Ahn et al. 2018). Therefore, FSP35 was used in this study.

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FIGURE 1

Nineteen johnsongrass cultivars inoculated after panicle emergence showed clear acervuli formation as early as 5 days postinoculation (dpi), whereas acervuli were not observable in seven cultivars up to 4 weeks postinoculation. Microscopic images (right column) for examples of lesions were magnified 10-fold and cropped, and macroscopic images are displayed in the left column: 1 = macroscopic image of SH1048; 2 = microscopic image of SH1048; 3 = macroscopic image of SH1136; 4 = microscopic image of SH1136; 5 = macroscopic image of SH1030; 6 = microscopic image of SH1030; 7 = macroscopic image of SH1426; and 8 = microscopic image of SH1426. The images were taken at 10 dpi with confirmation of numerous acervuli formation on 19 cultivars including SH1048 and SH1136.

Protocol

Twenty-six cultivars of johnsongrass (Supplementary Table S1) were provided by Jacob Barney (Virginia Tech University) in the form of rhizomes that were transplanted into plastic round pots filled with Sungro professional growing mix and grown in a greenhouse in College Station, TX, U.S.A. All the johnsongrass cultivars were inoculated at growth stages 2 (four to five leaves), 3 (seven to eight leaves), and 6 (post panicle emergence). A total of eight plants for growth stages 2 (four to five leaves) and 3 (seven to eight leaves) were inoculated in four independent replications. A total of three plants for growth stage 6 (post panicle emergence) were inoculated in three independent replications. *C. sublineola* isolate FSP35 was grown on Petri plates of half-strength potato dextrose agar for 10 to 14 days. Each culture plate was flooded with water, and the conidia were loosened with a sterile spatula. The loosened conidia were collected in sterile water and diluted. The spores were diluted to a concentration of $\sim 1 \times 10^6$ conidia/ml with distilled water, and two to three drops of TWEEN 20 were added as a wetting agent. The conidia concentration was measured by using a hemocytometer. Most of the johnsongrass cultivars were sterile under our greenhouse conditions. Two plant sets were prepared for each spray inoculation. The inoculated plants were either immediately covered with plastic bags for 1 week and/or misted for 20 s per hour in order to prevent desiccation of inoculum. Inoculated leaves showing potential signs of lesions were collected and brought to the laboratory every week from week 1 to week 4 postinoculation. Leaves were observed under an Olympus BX60 microscope to confirm acervulus formation, which was used as a

TABLE 1
Compatibility of *Colletotrichum sublineola* isolate FSP35 to 26 johnsongrass cultivars^a

Accession	Score	Timing of acervuli formation conformation
SH1002	3.33 ± 0.58	5 dpi
SH1030	2 ± 0	
SH1048	3.33 ± 0.58	10 dpi
SH1094	3 ± 1	14 dpi
SH1104	3.33 ± 0.58	10 dpi
SH1116	2.67 ± 0.58	10 dpi
SH1126	2 ± 0	
SH1136	3 ± 0	10 dpi
SH1152	3 ± 0	9 dpi
SH1154	3.67 ± 0.58	10 dpi
SH1165	3 ± 0	15 dpi
SH1201	3 ± 0	10 dpi
SH1229	3.67 ± 0.58	9 dpi
SH1233	3.67 ± 0.58	10 dpi
SH1247	2.67 ± 0.58	20 dpi
SH1281	4.33 ± 0.58	10 dpi
SH1325	3.33 ± 0.58	10 dpi
SH1337	3 ± 0	5 dpi
SH1350	3.33 ± 0.58	10 dpi
SH1409	2 ± 0	
SH1426	2 ± 0	
SH1450	3 ± 0	5 dpi
SH1457	2 ± 0	
SH1484	3.33 ± 0.58	10 dpi
SH1490	2 ± 0	
SH1493	2 ± 0	

^a Nineteen out of 26 johnsongrass cultivars formed acervuli by 4 weeks postinoculation. Mean score ± SD was generated based on three trials of greenhouse inoculation by JMP Pro 14. dpi = days postinoculation.

criterion for compatibility of host–pathogen relationship (Prom et al. 2009). The phenotypic data obtained from greenhouse inoculation were statistically analyzed by using Student's *t* test and Wilcoxon rank-sum test with JMP Pro 14.

Greenhouse Inoculation Results

Lesions and hypersensitive responses were found on leaves inoculated at growth stages 2 (four leaves) and 3 (eight leaves). Compared with upper leaves, lower leaves showed obvious damage such as discoloration, hypersensitive responses, and lesions. Lesion sizes varied from 1 mm to a few centimeters (1 mm to 5 cm diameter) among the 26 cultivars inoculated at the growth stage 2 or 3. However, no acervulus formation was confirmed on eight plants from four independent spray inoculations (two sets per trial). Likewise, when johnsongrass plants were spray inoculated after panicles were exposed (growth stage 6), hypersensitive responses and lesions were found on the inoculated surfaces. Lesions with clear acervulus formation were confirmed in 19 cultivars as early as 5 days postinoculation (dpi) (Fig. 1; Table 1). Although lesions were formed on all cultivars of johnsongrass, acervuli were found on 19 out of 26 cultivars of johnsongrass tested. Phenotypic responses to *C. sublineola* (FSP35) varied among the inoculated cultivars. For comparative purposes, a 1 to 5 scale for anthracnose disease assessment was used: 1 = no symptoms; 2 = hypersensitive reaction with reddening or red spots; 3 = infected bottom leaves

with acervuli; 4 = middle to bottom infected leaves with acervuli; and 5 = infected plant including flag leaf with acervuli (Erpelding and Prom 2004; Prom et al. 2016), with the results shown in Table 1.

A Role for Dhurrin

When plants were spray inoculated following emergence of the panicle, acervulus formation was confirmed in 19 cultivars as early as 5 dpi. The results confirm johnsongrass can serve as an alternate host of *C. sublineola* under the conditions tested, which included a favorable environment for pathogen infection, a strongly virulent isolate of *C. sublineola*, and nearly mature johnsongrass plants. Although the results could differ under field conditions, Tsedaley et al. (2016) found strong positive correlations between anthracnose severity and sorghum crop stage, and the same growth stage effect applied here for cross-infection to johnsongrass.

Sorghum biosynthesizes and accumulates the cyanogenic glycoside dhurrin during grain development from the early phase of development, reaching maximum amounts of dhurrin in 25 days after pollination and reducing to negligible amounts in mature grain (Nielsen et al. 2016). The site of synthesis of dhurrin shifts from leaves to stems during plant development (Busk and Møller 2002). Seedling stage resistance of sorghum plants to pathogens has been attributed to *C. graminicola* (now *C. sublineola*) in plants less than 5 weeks of age (Nicholson et al. 1988). Likewise, seedling-stage resistance was attributed to the presence of dhurrin. However, proof of a role in preventing anthracnose has been questioned due to its ability to detoxify the hydrogen cyanide breakdown product of dhurrin (Nicholson et al. 1988). Although correlation between developmental stages of johnsongrass and the dhurrin level has not been studied to our knowledge, dhurrin has been extracted from rhizomes (Nicollier et al. 1983). Therefore, as in sorghum, dhurrin could be induced as a defense response mechanism that involves production of phytoalexins in the early growth stages of johnsongrass, and as a result, johnsongrass cultivars at growth stages 2 and 3 were resistant, whereas most of the cultivars at growth stage 6 were susceptible to *C. sublineola* isolate FSP35.

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