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Stalk rot fungi affect grain sorghum yield components in an inoculation stage-specific manner

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ABSTRACT

Although stalk rots are among the most prevalent and destructive diseases of sorghum, no systematic crop loss assessment has been reported for these diseases under controlled-inoculations. The objective of this study was to assess the impacts of Fusarium stalk rot and charcoal rot on grain sorghum yield components when plants were inoculated at two growth stages (GS). Four genotypes were field evaluated against three *Fusarium* spp. (*F. thapsinum*, *F. proliferatum*, *F. andiyazi*) and *Macrophomina phaseolina*. Inoculations were performed at GS1 (30 d after emergence) and GS3 (14 d after flowering). Panicles were harvested at physiological maturity and assessed for total seed weight (TSW), 100-seed weight (100-SW), and total seeds per panicle (TSP). The total number of reproductive sites and unfilled spikelets were counted per rachis (TRSR and NUSR, respectively) and panicle (TRSP, NUSP) bases. Length and nodes crossed by the lesion in split stems were measured to evaluate disease severity. Pathogens significantly reduced TSW in comparison to the control at both GS1 and GS3. The four pathogens, on average, caused greater TSW reductions when inoculated at GS1 (52%) than at GS3 (37%). All pathogens reduced TSP upon GS1 inoculation and 100-SW upon GS3 inoculations. All pathogens significantly reduced seed set percentage when plants were inoculated at GS1 while inoculations at GS3 did not have a significant impact. GS1 inoculations significantly decreased TRSP, demonstrating pathogen interference with head formation resulting in smaller heads than control. Although inoculations at GS1 had a greater impact on yield, inoculations at GS1 and GS3 did not significantly differ in disease severity. This study revealed inoculation stage-specific effects of stalk rot pathogens on yield parameters and provided insights into key yield traits to be emphasized in sorghum breeding programs to produce stalk rot tolerant sorghum genotypes.

1. Introduction

Stalk rots are among the most ubiquitous diseases of sorghum (*Sorghum bicolor* L. Moench) worldwide (Zummo, 1984; Tesso et al., 2010). Infected plants are characterized by injured cortical and vascular tissue in the root and stalk systems that leads to impaired nutrient and water absorption and translocation (Hundekar and Anahosur, 1994). Bandara et al. (2016a,b) have shown that stalk rot fungi affect grain sorghum leaf chlorophyll content in a genotype- and growth stage-dependent manner. Under extreme conditions, stalk rot diseases can cause lodging due to the poor stalk strength in the infected area (Zummo, 1984). Prolonged water deficit stress, high temperature, and unbalanced mineral nutrition have been reported to predispose sorghum plants to stalk rot diseases (Dodd, 1980; Seetharama et al., 1987). There are two stalk rot diseases of sorghum, Fusarium stalk rot, caused by *Fusarium* spp., and charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goidanich (Tarr, 1962). Typically, infection by *Fusarium* spp. produces red colored lesions while *M. phaseolina* produces dark-colored lesions at the site of infection.

Although the reaction of sorghum genotypes to stalk rot diseases can vary based on the causal organism (Tesso et al., 2005), *Fusarium thapsinum* Klittich, Leslie, Nelson & Marasas is considered as the most aggressive stalk rot *Fusarium* pathogen of sorghum (Tesso et al., 2005, 2010, Tesso and Ejeta, 2011; Leslie et al., 2005). Charcoal rot is one of the most widespread and destructive stalk rot diseases of sorghum (Mughogho and Pande, 1984). Therefore, screening sorghum germplasm with several stalk rot fungi is critical

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for a better understanding of the pathogen dynamics involved and to recommend sorghum genotypes for multiple environments where different stalk rot fungi predominate.

Bandara et al. (2016a,b) have reported the negative impacts of stalk rot diseases on important biofuel traits of sweet sorghum under controlled inoculations. Although no systematic quantitative crop loss assessment results have been described for stalk rot diseases under controlled-inoculations for grain sorghum, standability and grain weight have been recognized to be affected by stalk rot fungi, which contribute to yield losses in sorghum (Tesso et al., 2012). Zummo (1980) reported that stalk rots slow down or inhibit the grain-filling process and result in shriveled seeds. Anahosur and Patil (1983) have attributed charcoal rot-mediated sorghum seed weight losses to the varying levels of lodging caused by *M. phaseolina*. Seetharama et al. (1991) have reported that there were no simple correlations between sorghum yield or yield components with stalk rot incidence. However, their conclusions were based upon a study conducted under natural inoculation. Moreover, the observed stalk rot incidences were attributed to *M. phaseolina*, based on symptomatology. Moreover, a few publications have provided information concerning the plant growth stage at which stalk rot infections occur and possible impacts they could have on yield. For example, Reed et al. (1983) and Jardine and Leslie (1992) reported that most stalk rot pathogens colonize the stalk and incite disease during the "post-flowering" stages. Khune et al. (1984) indicated that stalk rot pathogens are found in host tissues at various growth stages.

Eastin and Sullivan (1974) described a simple development stage terminology suitable for yield and yield components of grain sorghum according to the following growth stages: (i) the vegetative period from planting to panicle initiation (GS1); (ii) the reproductive period from panicle initiation to flowering (GS2); and (iii) grain filling from flowering to physiological maturity (GS3). The number of seeds per panicle is physiologically determined during the second growth stage (GS2) when floret number is set in the developing panicle (Eastin et al., 1999; Maiti and Bidinger, 1981). The yield components of sorghum include the number of panicles per square meter, number of seeds per panicle, and seed weight, which are defined as the first, second, and third yield components, respectively (Maman et al., 2004). As the second yield component directly relates to GS2, any biotic and/or abiotic stress that prevails before or at the onset of this stage could have serious second yield component impacts. Similarly, since seed filling is predominantly associated with GS3, stresses occurring at this stage can impact the third yield component. Therefore, the objective of this study was to determine the effects of multiple stalk rot pathogens and the stage of pathogen infection on the yield and yield components of selected grain sorghum genotypes. We hypothesize that different stalk rot fungi and the growth stage (GS1 and GS3) at which infection occurs have differential effects on sorghum yield, in general, and upon the second and third yield components, in particular.

2. Materials and methods

2.1. Sorghum genotypes and fungal isolates

Sorghum genotypes, SC599R (Fusarium stalk rot and charcoal stalk rot resistant, breeding line), BTx3042 (Fusarium stalk rot and charcoal rot susceptible, breeding line), 84G62 (Dupont Pioneer, hybrid), and DKS37-07 (Dekalb, hybrid) were used. Pioneer 84G62 and DKS37-07 are classified as charcoal rot tolerant and are commonly grown hybrids by producers in the state of Kansas, USA. The three *Fusarium* spp. used in this study, *F. thapsinum*, *F. proliferatum*, and *F. andiyazi*, were previously isolated from infected stalks by the Row Crops Pathology Lab at Kansas State University from local sorghum fields. A portion of the translation elongation factor (TEF-1α) gene of these isolates was PCR amplified with ef1 (forward) and ef2 (reverse) primers and subsequently sequenced (Geiser et al., 2004). Sequence information was used as a query for comparison with the NCBI and FUSARIUM-ID databases using BLAST algorithm to confirm the species identity. The *M. phaseolina* isolate (r144) was provided by Dr. Gary Odvody, Texas A&M AgriLife Research and Extension, Corpus Christi, Texas.

2.2. Field experiments

Field experiments were carried out during the 2013 and 2014 growing seasons at the Kansas State University agronomy research farm in Manhattan (39.22°N, 96.60°W) and Ashland (39.13°N, 96.62°W), Kansas, respectively (hereafter referred to as the two environments) under rainfed conditions. Field preparation, planting, and crop maintenance were conducted according to standard procedures for sorghum. Seeds were treated with fungicide (ethyl mercaptan (Captan), two mL kg^{-1} seed) and planted in rows of 5 m length $\times$ 0.75 m width (whole plot). The treatment structure was a $4 \times 2 \times 5$ factorial where factors one, two, and three consisted of four sorghum genotypes, two stages of inoculation, and four pathogens and mock-inoculated control, respectively. The field study was arranged in a split-split-plot design with randomized complete blocks. Genotypes were assigned to the whole-plot unit and stage of inoculation to the sub-plot unit (2.5 m length $\times$ 0.75 m width). Pathogen and control treatments were assigned to the sub-sub-plot unit (0.5 m length $\times$ 0.75 m width). There were three subsample plants (= observational unit) in each pathogen or mock-inoculated control treatment. For each environment, there were three replicated blocks.

2.3. Inoculum preparation, artificial inoculation, and measuring yield parameters

Inoculum preparation was performed according to the methods described by Bandara et al. (2015) and the protocol described therein for *F. thapsinum* inoculum preparation was also used to prepare *F. proliferatum* and *F. andiyazi* inocula. GS1 inoculations were performed at 30 days after seedling emergence for all genotypes when seedlings showed 7 to 10 leaf collars (ligules) (before flag leaf emergence). GS3 inoculations were performed at 14 days after flowering. At both stages, plants were inoculated by injecting 0.1 mL of inoculum at a concentration of 2×10^6 conidia or mycelium fragments per mL into the basal internode of the stalk. Conidia were used for *F. thapsinum*, *F. proliferatum*, and *F. andiyazi* inoculations. Hyphal fragments were used for *M. phaseolina* since this fungus does not typically produce spores. Phosphate-buffered saline (PBS) was used for the mock-inoculated control treatment. Inoculated plants were harvested at 110 days after planting (after all four genotypes attained physiological maturity). Panicles were separated from plants. The total number of rachis per panicle (TRP), total seeds per rachis (TSR), and the number of unfilled spikelets per rachis (NUSR) were counted for each panicle. The total number of reproductive sites per rachis (TRSR) in each panicle was computed by adding TSR and NUSR. These data were used to calculate the total seeds per panicle (TSP), number of unfilled spikelets per panicle (NUSP), total number of reproductive sites per panicle [TRSP = NUSP + TSP], and seed set percentage [SSP = (TSP/TRSP) $\times$ 100]. Panicles were then dried for 10 d at 40°C and threshed to measure the total seed weight per panicle (TSW). 100-seed weight (100-SW) was computed using the equation, (TSW $\times$ 100)/TSP. Stems were split longitudinally to measure lesion length (cm) and the number of nodes crossed by the lesion.