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Deanna L. Funnell-Harris
University of Nebraska-Lincoln, Deanna.Funnell-Harris@ars.usda.gov

Patrick M. O'Neill
University of Nebraska-Lincoln

Scott E. Sattler
University of Nebraska-Lincoln, Scott.Sattler@ars.usda.gov

Melinda K. Yerka
University of Nebraska-Lincoln, myerka@unl.edu

Response of sweet sorghum lines to stalk pathogens *Fusarium thapsinum* and *Macrophomina phaseolina*

Deanna L. Funnell-Harris, Patrick M. O'Neill, Grain, Forage and Bioenergy Research Unit (GFBRU), United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and Department of Plant Pathology, University of Nebraska, Lincoln 68583-0919, **Scott E. Sattler** and **Melinda K. Yerka**, GFBRU, USDA-ARS and Department of Agronomy and Horticulture, University of Nebraska, Lincoln

Corresponding author: Deanna L. Funnell-Harris

Email: Deanna.Funnell-Harris@ars.usda.gov

Abstract

Funnell-Harris, D. L., O'Neill, P. M., Sattler, S. E. and Yerka, M. K. Response of sweet sorghum lines to stalk pathogens *Fusarium thapsinum* and *Macrophomina phaseolina*. Sweet sorghum [*Sorghum bicolor* (L.) Moench] has potential for bioenergy. Itis adapted to a variety of U.S. locations and the extracted juice can be directly fermented into ethanol. However, little research on fungal stalk rots, diseases that pose serious constraints for yield and quality of juice and biomass, has been reported. A greenhouse bioassay was designed to assess charcoal rot (*Macrophomina phaseolina*) and Fusarium stalk rot (*Fusarium thapsinum*) in plants at maturity, the developmental stage at which these diseases are manifested. Multiple plantings of a susceptible grain line, RTx430, were used as a control for variation in flowering times amongst sweet sorghum lines. Lesion length measurements in inoculated peduncles were used to quantify disease severity. Sweet sorghum lines 'Rio' and M81E exhibited resistance to *F. thapsinum* and *M. phaseolina*, respectively, and in contrast, line 'Colman' exhibited susceptibility to both pathogens. Lesion development over time in Colman was monitored. These results will enhance molecular and biochemical analyses of responses to pathogens, and breeding stalk rot resistant sweet sorghum lines.

Sorghum [*Sorghum bicolor* (L.) Moench] has a vast diversity of germplasm, which is primarily being used for food and feed. However, several bioenergy platforms, grain sorghum (starch-based bioethanol), forage sorghum (cellulose-based bioethanol and thermal conversion) and sweet sorghum (sugars) are being used or are under development (Carpita and C., 2008; Upadhyaya et al., 2009; Wu et al., 2010; Zegada-Lizarazu and Monti, 2012). Similarly to its close relative, sugarcane (*Saccharum* spp.), the juice of sweet sorghum can be extracted and directly fermented into ethanol. Unlike sugarcane, sweet sorghum can be widely produced across the United States (Keeney and DeLuca, 1992; Smith et al., 1987). Sweet sorghum was traditionally used for small-scale syrup or sugar production, and, thus was bred for stalk height (biomass), juice volume, sugar content and juice extractability (Eggleston et al., 2013; Teetor et al., 2011; Wang et al., 2009). These traits also allowed sweet sorghums to be amenable for feeding ruminant animals plants or bagasse (biomass after juice extraction) (Smith and Frederiksen, 2000; Whitfield et al., 2012).

Little research on fungal stalk, foliar and root diseases of sweet sorghum has been reported (Dogget, 1988; Zummo, 1971; Zummo, 1986; Zummo and Broadhead, 1984). These diseases pose a serious constraint for yield and quality of sweet sorghum juice and bagasse (Funnell-Harris et al., 2014; Rajewski and Francis, 1991; Tesso and Ejeta, 2011). In particular, stalk diseases can reduce biomass and are associated with lodging, which reduces the harvestable yield (Bean et al., 2013; Funnell-Harris et al., 2014; Miron et al., 2005; Rajewski and Francis, 1991; Tesso et al., 2005).

Control of sorghum stalk diseases has been challenging, due to the diversity of fungi with relatively broad host ranges that are responsible for these diseases (Jardine and Leslie, 1992;

Saleh et al., 2010; Su et al., 2001; Tesso et al., 2010). Plant breeding has focused on identifications of QTLs and traits associated with increased resistance or tolerance. These traits include drought tolerance and post-flowering non-senescence, a trait called "stay-green" (Borrell et al., 2014; Tenkouano et al., 1993; Tesso et al., 2005). Sweet sorghum breeding has focused on stalk traits, such as biomass, juice and sugar content, that increase usability for bioenergy (Audilakshmi et al., 2010; Lv et al., 2013; Shiringani et al., 2010). There are no known publications on breeding sweet sorghum for increased resistance or tolerance to stalk pathogens.

The present research focusses on two major sorghum stalk diseases, Fusarium stalk rot, and charcoal rot (Bramel-Cox and Claflin, 1989; Funnell-Harris et al., 2014; Jardine and Leslie, 1992; Odvody and Dunkle, 1979; Tesso and Ejeta, 2011; Tesso et al., 2010). The primary *Fusarium* spp. infecting sorghum stalks include *Fusarium andiyazi* Marasas, Rheeder, Lampr., K.A. Zeller & J.F. Leslie, *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *Fusarium thapsinum* (authoritative name, *Gibberella thapsina* Klittich**,** J.F. Leslie, P.E. Nelson & Marasas), and *Fusarium verticillioides* (authoritative name: *Gibberella fujikuroi* (Sawada) Wollenw.) (Funnell-Harris and Pedersen, 2008; Funnell-Harris et al., 2014; Tesso et al., 2010). These species cause infections that can result in deterioration of stalk pith cells, associated with senescence during grain development (Reed and Partridge, 1983). *Macrophomina phaseolina* (Tassi) Goid. causes infections that result in similar deterioration of the stalk, but also form dark sclerotia along the degraded vascular bundles of the colonized stalk and roots (Rao et al., 1980; Russin et al., 1995), and therefore it was given the name "charcoal rot."

Based on previous published research (Davila-Gomez et al., 2011; Pfeiffer et al., 2010) (http://www.ars-grin.gov/npgs/) of brix (indicating sugar level), six lines were chosen as breeding materials for further improvement of bioenergy sweet sorghum. Five lines, 'Theis,'

'Dale,' 'Wray,' M81-E and 'Colman' were previously reported to be resistant to lodging while the sixth line, 'Rio' has no known reports (https://npgsweb.ars-grin.gov/gringlobal/descriptordetail.aspx?id=69023), and there are no known reports of resistance or susceptibility to stalk rot pathogens for any of these six lines. Therefore, it was necessary to assess responses of these lines to stalk rot pathogens under controlled conditionsanda greenhouse assay was developed to accomplish this. There have been no previously published reports of sweet sorghum pathology assays under controlled conditions. To account for large variation in flowering time amongst the sweet sorghum lines, several successive plantings of a grain sorghum line susceptible to the pathogens were used for comparison (Funnell-Harris and Pedersen, 2008; Funnell-Harris et al., 2014; Funnell and Pedersen, 2006a) (*unpublished*). In this way, the following hypothesis was tested: Among sweet sorghum lines, differential responses to stalk pathogens can be identified.

Materials and Methods

Plant lines: Sorghum lines Colman, Dale, M81E, Rio, Theis, Wray, RTx430, SC599 and SC1154, were utilized in this study (Table 1). SC599 and SC1154 were obtained from the National Plant Germplasm System, (PI534163 and PI59572, respectively), and were previously reported to be resistant to *Fusarium* spp. and *Macrophomina phaseolina* (Tesso et al., 2005; Tesso et al., 2010), while RTx430 has previously demonstrated susceptibility to these pathogens (Funnell-Harris and Pedersen, 2008; Funnell-Harris et al., 2014; Funnell and Pedersen, 2006a) (*unpublished*). RTx430, SC599 and SC1154 are all "combine height." Grain used in assays was produced in greenhouses at University of Nebraska, Lincoln, Plant Growth Facilities.

Fungi and media: *Gibberella thapsina* isolate H03S-11-9 (Funnell-Harris et al., 2010) and *M. phaseolina* isolate MP01-001 (a kind gift from G. Odvody, Texas A & M Agrilife Research and Extension Center, Corpus Christi, TX) were maintained on one-half strength potato dextrose agar (PDA; made using potato dextrose broth (PDB), Becton, Dickinson and Co., Sparks, MD) amended with 100 µg ml^{-1} ampicillin (Sigma-Aldrich, St. Louis). *Gibberella thapsina* is referred to in the text by its more familiar name *F. thapsinum.* To prepare inoculum, five agar disks (5 mm diameter) from the growing edge of 4-day-old cultures on PDA were inoculated into 25 ml sterile PDB in 150 ml beakers with sterile toothpicks, previously treated to remove toxins and other inhibitors to fungal growth (Jardine and Leslie, 1992). The broth-and-toothpick cultures were incubated at room temperature (22 - 23º C) 10 days before use.

Peduncle inoculations: Two repetitions were planted beginning in early- (Repetition (Rep.) 1) and midsummer (Rep. 2) of 2013 (Table 1). Based on previous field or greenhouse observations, the sweet sorghum lines and resistant checks were planted at different times to compensate for differences in flowering times between lines and to attempt to synchronize flowering times. RTx430 was also planted four (Rep. 1) or five (Rep. 2) plantings per repetition (A – I) for use as susceptible checks for greenhouse conditions at various times throughout the season.

Seeds were sown into 25.4 cm-diameter pots containing pasteurized soil mix (one part sand, one part coarse vermiculite, one part top soil, and two parts shredded peat moss). Seedlings were culled to one plant per pot. Inoculations (Table 1) were conducted on plants at anthesis (defined as one-half the anthers exerted). Peduncles were probed with a surface-disinfested awl to form a shallow hole (2mm diameter), then a fungal-inoculated toothpick was inserted into the hole. Eighteen days following inoculation, the peduncle was split longitudinally and the length of

the red to purple discoloration (the lesion) was measured. Control inoculations with sterile broth were used to account for pigmentation commonly resulting from wounding in sorghum.

The experimental design was randomized incomplete block with at least 2 lines (RTx430 and at least one sweet sorghum or resistant check), three treatments (*F. thapsinum, M. phaseolina* and broth) with six replications, blocked by time of inoculation and location. The data were analyzed using the PROC MIXED procedure of SAS/STAT software (SAS and all other SAS Institute, Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc. in the United States and other countries) (SAS, 2002-2008). The results from RTx430 inoculations were used to make comparisons across inoculation dates and between treatments. For comparisons of results between treatments on the same inoculation date, the data were treated as randomized complete block (RCB) design with six replicate blocks and two repetitions of the entire experiment. Data sets were analyzed for Levene's homogeneity of variance (HoV) and appropriate adjustments were incorporated using the REPEATED/GROUP option of PROC MIXED (SAS, 2002-2008). Least squares means (LSM) and standard errors (SE) are reported.

Peduncle lesion development: Experiments were conducted to observe the initiation and development of lesions in peduncles of sweet sorghum line, Colman. Two assays were planted in early- (assay 1) and midsummer (assay 2) 2014. In assay 1, 72 Colman plants were grown and, peduncles were inoculated as described above with toothpicks incubated with one of the three treatments, either *F. thapsinum, M. phaseolina* or broth. After inoculation (time point 0 days), four plants of each treatment were assessed for lesion length on days 0, 1, 3, 7, 14 and 18 post inoculation. RTx430 also was grown but the plants did not reach anthesis at the same time as Colman so inoculations were not performed. The experimental design for assay 1 was RCB with

one line, three treatments and six time points per treatment in four replications blocked by location. Results of assay 1 were used to adjust time points for assay 2. For assay 2, 72 plants each of Colman and RTx430 were grown and inoculated; four plants of each line and treatment were assessed for resulting lesion length on days 0, 3, 6, 9, 13 and 16 post inoculation. The experimental design was RCB with two lines, three treatments and six time points per treatment and line in four replications, blocked by location in the greenhouse.

For each time point assay, regression analyses were performed in order to compare between treatments (time point assays 1 and 2) and between lines for a given treatment (time point assay 2). Data were analyzed using the PROC MIXED procedure of SAS/STAT software (SAS, 2002-2008). Each line for each assay was analyzed separately. Because lesion measurement is destructive, measurements at each time point were performed on a different set of plants. The KENWARD-ROGER option was specified for estimating degrees of freedom. Slope was estimated using the SOLUTION option. Confidence intervals for the slopes were set at $P = 0.05$. Comparisons between treatments in a given line (assays 1 and 2), or between lines with the same treatment (assay 2) were made using single degree of freedom contrasts. Slope estimates and SE are reported.

For comparison of lesion length at each time point for a given treatment and plant line, the PROC MIXED procedure of SAS/STAT software (SAS 2002-2008) was used. The KENWARD-ROGER option was specified for estimating degrees of freedom. For analysis of time course assay 1, a HoV statement was added to adjust for variance between treatments. LSM and SE are reported.

Results

Response of the susceptible check (RTx430) to *F. thapsinum* and *M. phaseolina* inoculated at different times in the greenhouse. The susceptible grain sorghum line, RTx430, was planted nine different times under greenhouse conditions (Table 1). All plants were inoculated at anthesis with identically-prepared fresh inoculum; there was no significant effect due to inoculation date ($P = 0.16$) (Table 2). Across all inoculation dates, mean lesion lengths (mm) were significant for treatment: inoculations with *F. thapsinum, M. phaseolina* and broth control were 62.4 ± 4.8, 45.2 ± 4.6 and 14.0 ± 4.6 mm, respectively ($P < 0.01$). The interactions of inoculation date with treatment were significant ($P = 0.04$) (Table 2). This interaction may be due to inoculations with *F. thapsinum*, which resulted in significantly greater mean lesion lengths late summer or early fall, than inoculations occurring during fall or winter (Table 3; comparisons in columns). Inoculation date had a less pronounced effect on mean lesion lengths of *M. phaseolina* inoculated plants, and did not significantly affect the broth control when comparing responses at different dates. Mean lesion lengths resulting from pathogen inoculations were not always significantly greater than the lengths from broth inoculation due to pigmentation resulting from wounding response of the plant (Table 3; comparisons across rows).

Responses of sweet sorghum lines to *F. thapsinum* and *M. phaseolina*. Peduncle inoculations were conducted as these assays yield consistent results with relatively few replications; therefore, these assays are valuable for screening several plant genotypes and inoculum treatments for responses to stalk pathogens (Funnell-Harris and Pedersen, 2008; Funnell-Harris et al., 2014). Sorghum plants were inoculated at anthesis with either *F. thapsinum* or *M. phaseolina* and compared with the susceptible check, RTx430, inoculated at the same time. The key test statistics and significance levels for main effects and their interactions are shown in

Table 2 and mean lesion lengths are illustrated in Figure 1. Results from sweet sorghum lines indicate that Colman is more susceptible to both pathogens and Wray is more susceptible to *F. thapsinum*, than RTx430 ($P \leq 0.01$), while Rio exhibits more resistance to *M. phaseolina* than RTx430 in this assay ($P = 0.05$) (Fig. 1A,B). In addition, SC1154 ("resistant check") appears to be highly susceptible to both pathogens ($P < 0.01$), following greenhouse peduncle wound inoculations. Also, the wound responses of the resistant checks, SC1154 and SC599, were significantly greater ($P < 0.01$) than those of RTx430 based on the broth control inoculation (Fig. 1C). Differences in response to wounding and the broth control in different sorghum lines have been previously observed (Funnell and Pedersen, 2006a). To better discern the response due to pathogen inoculation as opposed to wound response (broth control), differences between LSM of lesion lengths due to inoculation with each pathogen and length following inoculation with the broth control were determined (Table 4). This analysis confirmed that Colman was susceptible to both pathogens in this assay. Following this analysis, it was clear that mean lesion length resulting on Rio was statistically similar as that on the resistant check, SC599 (Figure 1A, Table 4). Difference of the mean lesion length resulting on M81E following inoculation with *M. phaseolina* was not significantly different than the broth control (Table 4). These analyses indicated that Rio may have resistance to *F. thapsinm* and M81E may have resistance to *M. phaseolina*, while Colman is highly susceptible to both pathogens.

Lesion development within the susceptible sweet sorghum line, Colman. Colman exhibited susceptibility to both pathogens, especially when comparing with responses of the resistant check, SC599, to *F. thapsinum* and *M. phaseolina* (Fig. 1, Table 4). Therefore, documenting the lesion development within peduncles of the highly susceptible line, Colman, was undertaken to understand disease progression. The purpose of these assays was to determine

the number of days after inoculation in which initiation of a visible lesion and significant expansion of the lesion were observed. Colman (assays 1 and 2) and RTx430 (assay 2) plants were wound-inoculated at anthesis with toothpicks incubated in *F. thapsinum* or *M. phaseolina* cultures, or in sterile broth as previously described. Plants were harvested and lesion lengths measured over a time course. Regression analyses demonstrated that lesions forming in pathogen-inoculated peduncles were expanding at a greater rate (mm day^{-1}) than the wound responses, which resulted from sterile broth inoculation (Table 5). In the case of *M. phaseolina* inoculations, lesion expansion was significantly faster ($P = 0.03$) within Colman *versus* RTx430 peduncles.

In time course assay 1, visible lesions developed between day 1 to day 3: no visible lesions were apparent at day 1, but lesions were apparent by day 3 on many peduncles in both fungal inoculation and control (Fig. 2A). Lesions appeared to expand rapidly from day 7 to 14 in Colman peduncles, and mean lesion lengths were significantly greater than those of earlier time points for both pathogens ($P < 0.01$) at day 14. For time course 2, adjustments were made based on results of time course 1: an additional time point was added between 3 and 14 days and the susceptible check, RTx430, was included. By day 3, the presence of lesions was visible in both lines with both fungal inoculations in this assay (Fig. 2B). Lesions expanded from days 9 to 13 for both fungal treatments in Colman; lesion lengths were significantly greater at day 13 than the earliest time point ($P \leq 0.01$). Lesion expansion appeared to occur earlier, from days 6 to 9, in RTx430 peduncles, compared to Colman peduncles; hence, mean lesion lengths were significantly greater at day 9 than day 0 ($P \leq 0.01$) in RTx430. The lesions rapidly expanded between days 13 and 16 following inoculation with *F. thapsinum*; mean lesion length was significantly greater than all other time points ($P < 0.01$) at day 16 in RTx430 (Fig. 2B). In this

assay, there were no significant differences in mean lesion lengths of broth control inoculations between lines ($P = 0.12$; Table 4) or different time points within a line ($P \geq 0.21$; Fig. 2B).

Discussion

This study determined that sweet sorghum lines differentially respond to the stalk pathogens, *F. thapsinum* and *M. phaseolina* that cause Fusarium stalk rot and charcoal rot, respectively. Rio exhibited greater resistance or tolerance to *F. thapsinum* similar to the resistant check, SC599. M81E had similar lesion lengths following inoculation with *M. phaseolina* as observed with the broth control (wound response). Colman was highly susceptible to both pathogens (Table 4, Figure 1). Thus, resistance to both stalk pathogens exists in sweet sorghum lines; therefore, it may not be necessary to use other sorghum germplasm to develop improved sweet sorghum lines with resistance to both pathogens (Lv et al., 2013; Pfeiffer et al., 2010; Shiringani et al., 2010).

Although controlled greenhouse assays with sweet sorghum have been reported (Luo et al., 2012; Nimir et al., 2015), there have been no reports of pathology assays conducted on mature plants to our knowledge. Because stalk rots manifest themselves at anthesis (Tenkouano et al., 1993; Tesso et al., 2005; Tesso et al., 2010), it was necessary to grow plants to near full maturity. Both photoperiod and temperature affect flowering in sorghum (Major et al., 1990; Prasad et al., 2008; Yanase et al., 2008). Therefore, multiple plantings of a susceptible grain sorghum line RTx430 were included in the current study, as a benchmark for the greenhouse conditions at the time the sweet lines were inoculated (Boedo et al., 2012; Buttner et al., 2004; Padley et al., 2008). However, lesion lengths resulting from inoculation with *F. thapsinum* were affected by greenhouse conditions, which had been previously observed (Funnell-Harris and

Pedersen, 2008; Funnell-Harris et al., 2010; Funnell-Harris et al., 2014; Paul and Munkvold, 2005; Pedersen and Morrall, 1994; Shpialter et al., 2009). Conducting repetitions of the experiment at different times of the year addressed the potential effects of seasonality on stalk rot development and associated plant responses (Dorrance and Inglis, 1997; English and Beuselinck, 2000). Additionally, two lines, SC599 and SC1154, previously reported to be resistant to both *F. thapsinum* and *M. phaseolina* in field studies (Tesso et al. 2005, 2010) were included. During the present greenhouse study, SC599 exhibited responses consistent with those previously reported, but SC1154 appeared to be susceptible to the two pathogens, even when the relatively large wound response was taken into consideration (Table 4, Fig. 1). This inconsistency may be due to differences in controlled conditions in the greenhouse *versus* conditions present in the field, as well as differences in inoculum and delivery (syringe injection of conidia in the field studies *versus* wound inoculation with mycelia in the present study) and location of inoculation (older tissue at the base of the stalk in previous field studies *versus* peduncle in the present study) (Tesso et al. 2005, 2010).

Lesion development within the susceptible sweet sorghum line Colman gave hints to aspects of colonization that could be restricted in the more resistant lines. For example, initial infection could be delayed or expansion of the lesion could be limited (Dita Rodriguez et al., 2006; Dugan et al., 2011; Onfrey et al., 2007). The approximate timing of lesion initiation (2 to 3 days) and lesion expansion (9 to 13 days) were determined within Colman peduncles inoculated either by *F. thapsinum* or *M. phaseolina*. “Lesions” were defined as pigmentation along the length of the peduncle in this study, which is a plant response to wounding and infection (Funnell-Harris et al., 2013; Funnell and Pedersen, 2006b). Response to wounding or to fungal infection was not yet visible by day 1, but it was clearly visible by day 3 following inoculation.

Timing of this response is consistent with previous observations where seedling epicotyls were inoculated with *F. thapsinum, F. proliferatum* or the non-pathogenic *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker (Huang and Backhouse, 2005; Lo and Nicholson, 1998). Because the peduncle inoculation assay was destructive, it prevented assessment of lesion development of an individual plant over the time course. Nonetheless, combined analyses of variances of slopes and mean lesion lengths at individual time points allowed conclusions to be drawn as to initiation and expansion of lesions within Colman peduncles. Lesion expansion in another susceptible line, RTx430, began sooner and expanded rapidly 13 to 16 days after *F. thapsinum* inoculation. Further investigations will be needed to establish the timing of lesion development within RTx430 peduncles. Defining the stages of lesion development within the susceptible Colman peduncles will facilitate the discovery of molecular and biochemical responses to stalk pathogens during lesion development in susceptible and resistant sweet sorghum lines.

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Table 1. Field observations for plant heights and days to anthesis; planting and inoculation (at anthesis) dates for two repetitions of peduncle inoculations assays.

Lines	Field observations[a]		Repetition 1		Repetition 2	
	Days to anthesis	Height (cm)	Planting date	Inoculation date	Planting date	Inoculation date
Sweet sorghums						
'Colman'	84.6	300.0	8/14/2013	10/11/2013	10/29/2013	1/22/2014
'Dale'	94.8	331.7	6/26/2013	9/5/2013	9/18/2013	12/16/2013
M81E	95.2	343.3	6/26/2013	9/5/2013	8/21/2013	11/25/2013
'Rio'	91.6	340.0	8/19/2013	9/26/2013	9/18/2013	11/25/2013
'Theis'	99.4	343.3	6/26/2013	9/26/2013	8/21/2013	11/25/2013 (16) 12/16/2013 (2)
'Wray'	91.0	325.0	8/21/2013	10/21/2013	10/29/2013	3/5/2014
Resistant checks						
SC599	ND[b]	122[c]	8/8/2013	10/21/2013	11/1/2013	2/10/2014
SC1154	ND	ND[d]	8/8/2013	10/11/2013	11/1/2013	2/10/2014 (16) 3/2/2014 (2)
Susceptible check						
RTx430	81.6	123.3	7/10/2013 7/24/2013 8/7/2013	9/5/2013 (A) 9/26/2013 (B) 10/11/2013 (C)	9/10/2013 (E) 9/24/2013 (F) 10/9/2013 (G)	11/25/2013 12/16/2013 1/22/2014

	8/21/3013	10/21/2013 (D)	10/29/2013 (H)	2/10/2014
			11/20/2013 (I)	3/5/2014

[a]Field observations were made during summer, 2014. Average plant height was estimated at maturity. Measurements on each of five plots were rounded to the nearest 5 cm. Means are shown; standard errors (SE) are 8.3. Days-to-anthesis was determined when the anthers from half the plants in a plot were exerted. Means were calculated based on three plots. Means are shown; SE are 1.8.

[b]"ND" denotes "not determined."

[c]Mean height of SC599 published in National Plant Germplasm System (NPGS) (http://www.ars-grin.gov/npgs/).

[d]In NPGS, SC1154 is listed as being "combine height," similar to other grain sorghums.

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Table 2. Analyses of variance of fixed effects (line, treatment and inoculation date) and interactions for inoculations of sweet sorghum lines and resistant checks with *Fusarium thapsinum*, *Macrophomina phaseolina* and control, as compared with the susceptible check, RTx430[a].

		Summary statistics from Type 3 tests of fixed effects						
Line	**Statistic**	**Line (L)**	**Trt (T)**	**Date (D)**	**L × T**	**L × D**	**T × D**	**L × T × D**
Susceptible check								
RTx430	F-value	na[b]	27.30	1.52	na	na	1.80	na
	P-value	na	<0.01	0.16	na	na	0.04	na
Sweet sorghum lines								
Colman	F-value	18.08	14.59	6.40	6.01	5.98	10.08	3.23
	P-value	<0.01	<0.01	0.02	0.01	0.02	<0.01	0.06
Dale	F-value	4.23	34.27	37.92	0.15	1.08	3.17	1.59
	P-value	0.05	<0.01	<0.01	0.87	0.31	0.06	0.22
M81-E	F-value	0.00	9.48	1.81	0.23	0.27	4.87	0.50
	P-value	1.00	<0.01	0.18	0.80	0.61	0.01	0.61
Rio	F-value	1.39	20.07	0.51	1.86	4.56	1.64	1.39
	P-value	0.24	<0.01	0.48	0.17	0.04	0.20	0.26
Theis	F-value	0.43	8.91	0.88	0.57	0.00	3.11	1.84
	P-value	0.51	<0.01	0.35	0.57	0.98	0.05	0.17
Wray	F-value	12.45	72.81	0.21	12.22	0.28	0.41	3.87
	P-value	<0.01	<0.01	0.65	<0.01	0.60	0.66	0.03
Resistant checks								

SC 599	F-value	9.64	49.23	0.79	1.53	0.16	3.97	5.61
	P-value	<0.01	<0.01	0.38	0.22	0.69	0.02	0.01
SC1154	F-value	54.31	12.06	0.74	0.24	0.01	1.25	0.99
	P-value	<0.01	<0.01	0.39	0.79	0.91	0.29	0.38

[a]Peduncles were inoculated at anthesis with toothpicks infested with one of the two fungi, or broth (control). Due to variabilities in flowering, the susceptible check, RTx430, was included at each inoculation date. The response variable was lesion length (mm), measured 18 days after inoculation.

[b]"na" indicates "not applicable."

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Table 3. Response of susceptible grain sorghum line RTx430 to peduncle inoculations with the stalk pathogens, *Fusarium thapsinum* and *Macrophomina phaseolina* or with broth control at nine times over six months under greenhouse conditions.[z]

	Treatments		
Inoculation dates	***F. thapsinum***	***M. phaseolina***	**Broth control**
9/5/2013 (A)	104.3a,l ± 13.5	64.5ab,m ± 13.5	10.2a,n ± 13.5
9/26/2013 (B)	108.2a,l ± 13.5	30.0b,m ± 13.5	17.7a,m ± 13.5
10/11/2013 (C)	63.8b,l ± 13.5	35.8ab,lm ± 13.5	18.7a,m ± 13.5
10/21/2013 (D)	58.7b,l ± 13.5	39.3ab,lm ± 13.5	4.7a,m ± 13.5
11/25/2013 (E)	49.6b,l ± 14.8	71.2a,l ± 14.8	7.3a,m ± 13.5
12/16/2013 (F)	42.6b,l ± 14.8	29.2b,l ± 13.5	12.8a,l ± 14.8
1/22/2014 (G)	33.0b,lm ± 16.6	61.7ab,l ± 13.5	20.8a,m ± 13.5
2/10/2014 (H)	54.4b,l ± 11.7	31.0b,lm ± 13.5	8.0a,m ± 13.5
3/5/2014 (I)	43.3b,l ± 14.8	44.0ab,l ± 14.8	25.6a,l ± 14.8

[z]Peduncles of RTx430 plants were wound-inoculated at anthesis with toothpicks incubated in a broth culture of *F. thapsinum, M.phaseolina* or sterile broth (control). Eighteen days later peduncles were split longitudinally and lesion lengths were measured. The first letters (a, ab or b) are for comparisons of mean lesion lengths from the same treatment across inoculation dates (column). The second letters (l, lm, m or n) are for comparisons of different treatments at the same inoculation date (row).

Comparisons with differing letters are significantly different at $P \leq 0.05$.

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Table 4. Difference in least squares means (LSM) of lesion lengths relative to the broth control for sorghum lines inoculated with *Fusarium thapsinum* or *Macrophomina phaseolina*.[a]

	F. thapsinum		*M. phaseolina*	
Line	**Difference in mean lesion lengths (mm) $LSM_{Ft\text{-}broth}$**	**Pr > t[b]**	**Difference in mean lesion lengths (mm) $LSM_{Mp\text{-}broth}$**	**Pr > t[b]**
Sweet sorghum line				
Colman	119.4	<0.01	48.9	0.07
Dale	61.6	<0.01	42.2	<0.01
M81-E	54.1	0.01	19.6	0.35
Rio	36.8	<0.01	20.9	0.05
Theis	39.6	0.03	21.1	0.24
Wray	80.7	<0.01	33.7	<0.01
Resistant checks				
SC1154	63.5	<0.01	28.3	0.09
SC599	36.7	<0.01	18.9	<0.01
Susceptible check				
RTx430	48.5	<0.01	31.2	<0.01

[a]Peduncles of plants were wound-inoculated at anthesis with toothpicks incubated with

F. thapsinum, *M. phaseolina* or broth control. Eighteen days later peduncles were split longitudinally and lesion lengths were measured.

[b]T-test if difference between LSM = 0.

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Table 5. Rate of lesion expansion (mm day^{-1}) from regression analyses of lesion lengths following inoculation with the stalk pathogens *Fusarium thapsinum* and *Macrophomina phaseolina* and the broth control.

Treatment	Assay 1[w]	Assay 2[x]	
	Colman	Colman	RTx430
F. thapsinum	6.90a[y] ± 0.91	5.40a ± 0.69	6.99a ± 1.87
M. phaseolina	3.41b ± 0.86	4.09a*[z] ± 0.41	2.31b ± 0.69
Control	1.29c ± 0.45	1.38b ± 0.33	0.77c ± 0.19

[w]Peduncles of Colman plants at anthesis were wounded with surface disinfested awls and inoculated with a toothpick incubated in a broth culture of each fungus or in broth, alone. On days 0, 1, 3, 7, 14 and 18, lesion lengths of four randomly chosen plants were measured; on day 1, no lesion was apparent so lesion length was assumed to be the awl diameter (2 mm). Mean lesion length at day 0 was also assumed to be 2 mm. Slope estimates and standard errors (SE) are shown.

[x]Peduncles of Colman and RTx430 plants at anthesis were inoculated as described for Assay 1. On days 0, 3, 6, 9 13 and 16, lesion lengths of four randomly chosen plants were measured. Mean lesion length at day 0 was assumed to be 2 mm. Slope estimates and SE, from comparisons within a line, are shown.

[y]Letters indicate comparisons of different treatments in a given line and assay (vertical columns). Mean slopes with differing letters are significantly different at $P \leq 0.05$.

[z]The asterisk indicates that the mean slope of lesion expansion on Colman following inoculation with *M. phaseolina* is significantly greater than that on RTx430.

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Figure legends

Figure 1. Mean lesion lengths on sweet sorghum (solid black bars) and resistant checks (solid white bars) as compared with the susceptible check (diagonal hatch marks) following wound inoculation with A. *Fusarium thapsinum*; B. *Macrophomina phaseolina*; and C. broth control. Peduncles of plants were inoculated with one of the fungi or with sterile broth. Eighteen days following inoculation, the peduncles were split longitudinally and the lengths of the resulting lesions were measured. Each sorghum line was inoculated at the same time as the susceptible line, RTx430. On the horizontal axis, letters following RTx430 indicate the replications within each repetition; inoculations with the same letter were inoculated at the same time. Mean lesion lengths and positive standard errors are shown. An asterisk indicates that the mean lesion length is significantly different ($P \leq 0.05$) than that resulting on RTx430.

Figure 2. Lesion development after peduncle inoculations in sweet sorghum line 'Colman' (A & B) and grain sorghum line RTx430 (B) with the stalk pathogens *Fusarium thapsinum* (top row of panels), *Macrophomina phaseolina* (middle row of panels), or broth control (bottom row of panels). Mean lesion lengths are illustrated; time points with differing letters are significantly different at $P \leq 0.05$. A. Colman plants (4) were harvested at 0, 1, 3, 7, 14 and 18 days after inoculation and lesion lengths were determined. At day 1, no visible lesions were observed; therefore lesion length was considered diameter of wound (2 mm); similarly at day 0, lesion was assumed to also be diameter of wound. Standard errors (SE) for *F. thapsinum* were 12.4, for *M. phaseolina*, SE were 10.2, and for broth control, SE were 5.9 (days 0, 1, 7 and 14) or 6.8 (days 3 or 18). B. Colman (blue) and RTx430 (red) plants (4 each) were harvested at 0, 3, 6, 9, 13 and 16

days after inoculation and mean lesion lengths were determined. At day 0, lesions were assumed to be diameter of wound (2 mm). SE were 12.4 for both lines, all treatments.

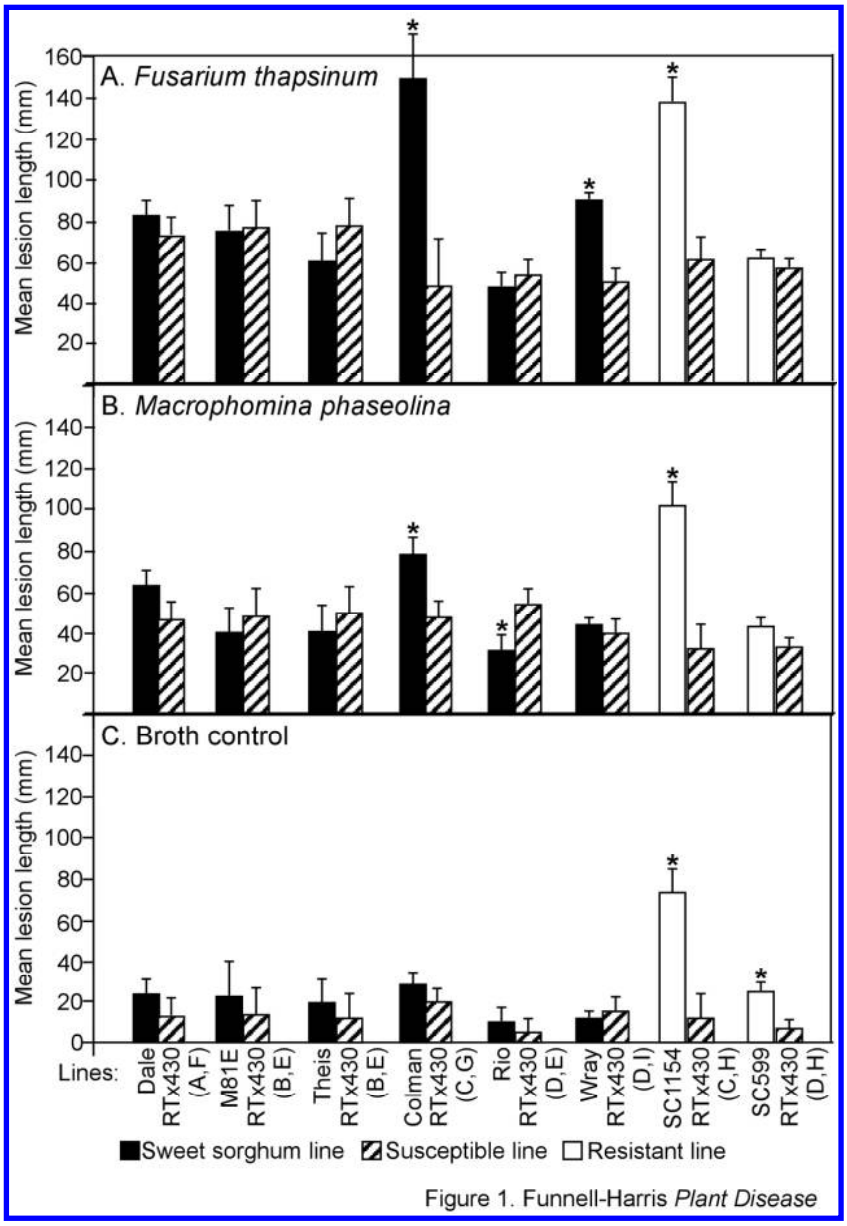

Figure 1. Funnell-Harris *Plant Disease*

151x220mm (300 x 300 DPI)

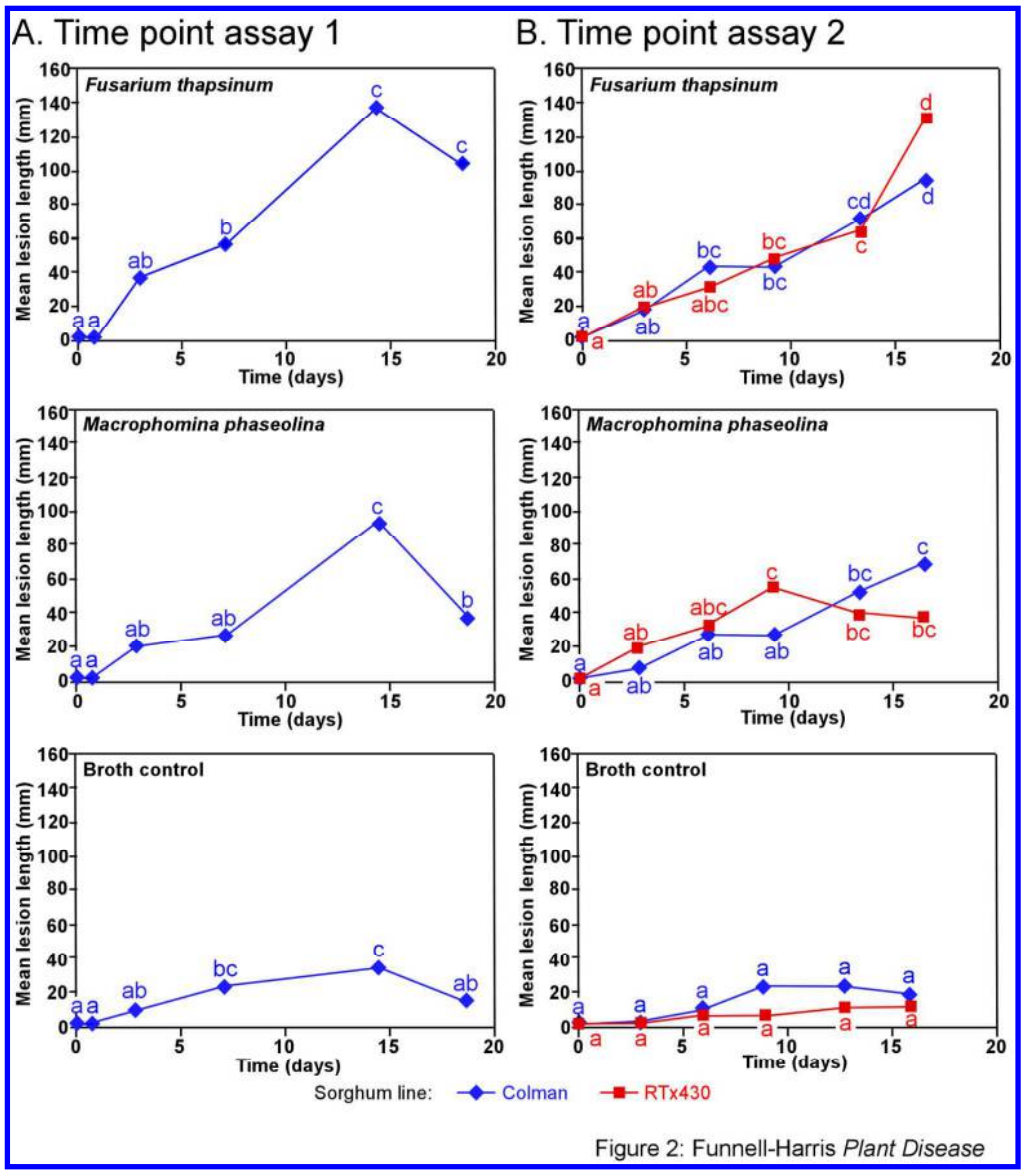

Figure 2. Lesion development after peduncle inoculations in sweet sorghum line 'Colman' (A & B) and grain sorghum line RTx430 (B) with the stalk pathogens Fusarium thapsinum (top row of panels), Macrophomina phaseolina (middle row of panels), or broth control (bottom row of panels). Mean lesion lengths are illustrated; time points with differing letters are significantly different at P ≤ 0.05. A. Colman plants (4) were harvested at 0, 1, 3, 7, 14 and 18 days after inoculation and lesion lengths were determined. At day 1, no visible lesions were observed; therefore lesion length was considered diameter of wound (2 mm); similarly at day 0, lesion was assumed to also be diameter of wound. Standard errors (SE) for F. thapsinum were 12.4, for M. phaseolina, SE were 10.2, and for broth control, SE were 5.9 (days 0, 1, 7 and 14) or 6.8 (days 3 or 18). B. Colman (blue) and RTx430 (red) plants (4 each) were harvested at 0, 3, 6, 9, 13 and 16 days after inoculation and mean lesion lengths were determined. At day 0, lesions were assumed to be diameter of wound (2 mm). SE were 12.4 for both lines, all treatments.

184x212mm (300 x 300 DPI)

Response of sweet sorghum lines to stalk pathogens *Fusarium thapsinum* and *Macrophomina phaseolina*

Deanna L. Funnell-Harris, Patrick M. O'Neill, **Scott E. Sattler** and **Melinda K. Yerka**

Interpretive summary:

Sweet sorghum [*Sorghum bicolor* (L.) Moench] has potential for bioenergy. This crop can be grown in several regions in the U. S. and the juice extracted from the stalks can be used directly in ethanol production. However, research is needed to determine whether stalk rot diseases pose serious problems on yield and quality of juice and biomass of sweet sorghum. We designed a greenhouse test to determine how sweet sorghum varieties respond to two major stalk diseases: charcoal rot and Fusarium stalk rot. The key to this experiment was to stagger plantings of a sorghum variety susceptible to both diseases. We determined that sweet sorghum varieties 'Rio' and M81E were resistant to Fusarium stalk rot and charcoal rot, respectively, while line 'Colman' was susceptible to both diseases. In addition to determining susceptibility of the sweet sorghum varieties, we documented how the two stalk rot diseases progressed over time in Colman. These results can be used to breed stalk rot resistant sweet sorghum varieties.