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ORIGINAL RESEARCH ARTICLE

Plant Genetic Resources

Performance of novel sorghum germplasm in Pennsylvania and their response to anthracnose

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Abstract

Sorghum (*Sorghum bicolor* L.) has the potential to become a widespread commercial feedstock crop in Pennsylvania, either in rotation with maize (*Zea mays* L.) or grown alongside it. In other locations where sorghum has been grown for a long time, it is attacked by *Colletotrichum sublineola* Henn. ex Sacc. & Trotter, a fungal pathogen that causes anthracnose (*Colletotrichum sublineola*) leaf blight (ALB), thereby diminishing yield. Field surveys were carried out in 2011, 2012, and 2016 to monitor the presence of *C. sublineola* in commercial sorghum fields in six Pennsylvania locations. Senescing, lower leaves developed lesions that yielded *Colletotrichum* sp., including isolates of *C. sublineola*. The pathogen was not recovered from field debris, and ALB symptoms were not observed on the younger leaves of plants. In preparation for widespread cultivation of sorghum in Pennsylvania, we evaluated the performance, in field and greenhouse tests, of 158 experimental lines and commercial hybrids, which had been improved in several states in the United States and in other parts of the world. Sources of resistance to ALB and other foliar diseases were discovered that should be useful in breeding programs targeted for Pennsylvania and for northeastern U.S. climatic conditions. Lines received from ICRISAT, especially ICSB94, showed the highest level of resistance in the field.

1 | INTRODUCTION

There has been an interest in the United States, including in the northeast, in the evaluation and promotion of sorghum (*Sorghum bicolor* L.) as a sustainable feedstock substitute for maize (*Zea mays* L.) (Kim & Day, 2011; Mathur, Umakanth, Tonapi, Sharma, & Sharma, 2017; Rao & Kumar, 2012). Sorghum utilizes water and nutrients, especially nitrogen, more efficiently than maize (Reddy & Reddy, 2003) and it is generally better adapted to environmental stresses (e.g., drought, salinity, heat) (House, 1985). This annual crop thrives on marginal lands and can be grown across the same climatic range as maize, using similar agronomic practices, making it a highly practical substitute (Rao, Umakanth, et al., 2013). Large-scale sorghum cultivation has been limited to the arid regions of the United States in the past, but it is now gaining popularity throughout the country. Diseases cause significant negative impacts on yield in current sorghum production areas in the United States, including Arkansas (TeBeest, Kirkpatrick, & Cartwright, 2004), South Carolina (Dowling,

Abbreviations: ALB, anthracnose leaf blight; ASR, anthracnose stalk rot; BLAST, Basic Local Alignment Search Tool; CI, confidence intervals; DS, disease severity; dpi, days post inoculation; ITS, internal transcribed spacer; NAM, nested association mapping; OA, oatmeal agar; PCR, polymerase chain reaction; PDA, potato dextrose agar; RCBD, randomized complete block design; RME, relative marginal effects

Schnabel, Williamson, Sekhon, & Zielinski, 2016), and Florida (Kucharek, 1992). In Pennsylvania, sorghum is a relatively new crop, grown mainly in the south-central part of the state, and it has not yet experienced widespread disease epidemics. However, as area under sorghum increases in the state, diseases may become a significant production-limiting factor in the future.

Introduction of sorghum into a maize–winter cereal–soybean rotation is likely to result in increased biotic stresses on both maize and sorghum (Craven & Nel, 2017; Schmidt, Mitchell, & Scow, 2019). Both crop species belong to the Poaceae, are evolutionarily closely related (Berhan, Hulbert, Butler, & Bennetzen, 1993; Devos & Gale, 2000), and are attacked by many of the same pests and pathogens. Anthracnose (*Colletotrichum sublineola*) leaf blight (ALB) and anthracnose stalk rot (ASR) are among the most important diseases of sorghum and maize in the United States and worldwide (Frederiksen, 2000; Mathur, Thakur, Neya, Marley, & Casela, 2003; Mughogho, 1983). *Colletotrichum sublineola* Henn. ex Sacc. & Trotter causes ASR and ALB in sorghum, with the latter being by far the more common and damaging disease of the two. This fungus is also a pathogen of weedy relatives Johnsongrass [*Sorghum halepense* (L.) Pers.] and shattercane (*Sorghum bicolor* L.). Its close relative *Colletotrichum graminicola* (Ces.) G. W. Wils. causes ALB and ASR in maize. Most studies agree that *C. sublineola* and *C. graminicola* are host-specific (Buiate et al., 2017; Jamil & Nicholson, 1987; Williams & Willis, 1963). However, there is also evidence that both species can colonize and sporulate on non-host plants under certain conditions including senescence or environmental stress (Chowdhury, 1936; Huguenin, Lourd, & Geiger, 1982; Lohman & Stokes, 1944; Venard & Vaillancourt, 2007a, 2007b; Wheeler, Politis, & Poneleit, 1974). Higher planting densities, water shortages, and global climate change are likely to exacerbate crop stresses.

Increasingly, farmers are switching from crop rotations to continuous no-till cultivation of maize for economic reasons. Continuous culture and no-till or reduced tillage have led to an increase in disease pressure, resulting in severe production losses in maize (Bergstrom & Nicholson, 1999; Kelly, 2017; Lipps, 1988; White, 1999). Similar practices in sorghum cultivation engender a potential for an increased inoculum density of *C. sublineola* because of its ability to survive and reproduce on weedy relatives (Xavier, Pfeiffer, Parreira, Chopra, & Vaillancourt, 2017), and to overwinter on crop debris (Bergstrom & Nicholson, 1999; Lipps, 1988). *Colletotrichum sublineola* is also able to colonize unwounded maize rind epidermal cells and wounded maize stalk tissues, and it can sporulate in those tissues once they senesce (Venard & Vaillancourt, 2007b). Thus, there is a possibility that *C. sublineola* could survive in maize crop debris in a sorghum–maize rotation, which would allow buildup of fungal population levels even more quickly. As area under sorghum cultivation increases, it is anticipated that there will be increased disease pressure on both maize and sorghum in Pennsylvania.

Core Ideas

- The anthracnose pathogen *Colletotrichum sublineola* was recovered from senescent sorghum leaves.
- *Colletotrichum graminicola*, a pathogen of maize, was also isolated from the senescing sorghum leaves.
- Resistant germplasm for incorporation into sorghum breeding programs was identified.

To protect our feedstock supply and maximize productivity in the future, we must have a better understanding of the dynamics of *C. sublineola* on sorghum to be able to make informed decisions regarding suitable accessions, crop rotation, and disease-management regimens. Our study is the first to investigate the frequency, diversity, and distribution of *C. sublineola* and other *Colletotrichum* species on sorghum in Pennsylvania. Our findings will help develop better recommendations for sorghum growers so they can manage and proactively prevent the buildup of inoculum and disease outbreaks. Our work also demonstrates the value of testing a broader range of germplasm, including more diverse sources of resistance, for the development of improved lines that will offer more durable and efficacious control of ALB. The goals of this research were: (a) to survey fields in Pennsylvania where sorghum has been grown for at least 2 yr for the occurrence of *Colletotrichum*; and (b) to study the resistance of a diverse collection of sorghum accessions to *C. sublineola* ALB when grown under a no-till regimen in rotation with maize in central Pennsylvania.

2 | MATERIALS AND METHODS

2.1 | Collection and identification of locally occurring *Colletotrichum* isolates

2.1.1 | Recovery of putative *Colletotrichum* isolates

Sorghum stubble and surface debris were collected from fields in three counties in southeastern Pennsylvania, in the early spring of 2011 and 2012 before planting (Figure 1). The locations were Martinsburg, Landisville, and Pillow. The stubble and debris were incubated under moist conditions and continuous light for 4 d and then examined for the presence of acervuli with setae characteristic of *Colletotrichum*. Spores

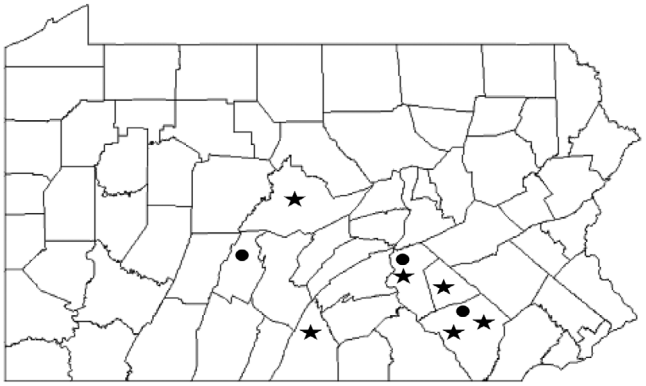

FIGURE 1 Map of Pennsylvania counties showing the collection sites. Circles indicate counties surveyed in 2011 and 2012 (Martinsburg in Blair County, Landisville in Lancaster County, and Pillow in Dauphin County), the stars indicate counties surveyed in 2016 (Russel E. Larson Agricultural Research Center at Penn State in Center County, Pillow in Dauphin County, State line in Bedford County, Mount Joy and Christiana in Lancaster County, and Lebanon in Lebanon County)

were isolated from acervuli and cultured on oatmeal agar (OA: 2% organic oat flour, 1.5% agar).

In the late spring of 2016, lesions on older lower leaves of sorghum plants that ranged from the V4 growth stage to flowering were collected from the original three locations, and three additional locations (Figure 1). Fragments of the symptomatic leaf tissues were surface sterilized and plated on half-strength potato dextrose agar (PDA) amended with ampicillin (100 mg L^{-1}). Plates were incubated for 24 h and then inspected for the presence of fruiting structures containing setae characteristic of *Colletotrichum*. Spores were isolated from the fruiting structures and cultured on OA. All fungal isolates were single-spored and stored on silica gel at −80 ˚C (Tuite, 1969). Single-spored isolates were morphologically characterized to confirm their similarity to *C. sublineola* (Jayawardena et al., 2016; Sutton, 1980).

2.1.2 | Internal transcribed spacer sequencing of putative *Colletotrichum* isolates

The identities of putative *Colletotrichum* isolates were confirmed by sequencing the rDNA internal transcribed spacer (ITS) region. Fungal DNA was prepared from single-spored isolates using the method described by Xavier, Mizubuti, Queiroz, Chopra, & Vaillancourt (2018). Amplicons were obtained by using the primer pair ITS4 and ITS5 (Crouch, Clarke, & Hillman, 2006; Innis, Gelfand, Sninsky, & White, 2012). The polymerase chain reaction (PCR) was carried out with GoTaq DNA polymerase (Promega Corporation) in a final reaction volume of 50 µl containing 50 ng of template DNA and 5 µM of each primer (Integrated DNA Technologies). The PCR cycling conditions consisted of 95 ˚C for 3 min, followed by 25 cycles consisting of denaturation at 95 ˚C for 30 s, annealing at 55 ˚C for 30 s; extension at 72 ˚C for 1 min; with a final extension at 72 ˚C for 10 min. Amplicons were purified using a StrataPrep PCR purification kit (Agilent Technologies) and cloned into a pMiniT vector using the NEB PCR Cloning Kit (New England Biolabs Inc.). Two clones from each isolate were sequenced using vector primers (Eurofins Genomics). After trimming to remove vector and low-quality sequence, consensus sequences were used to identify each strain by BLAST (Basic Local Alignment Search Tool) comparison against the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov), and/or by pairwise sequence comparison against the MycoBank database (http://www.mycobank.org). Sequences were deposited in Genbank (https://www.ncbi.nlm.nih.gov) and their accession numbers are listed in Table 1.

2.1.3 | Pathogenicity assays of *Colletotrichum* isolates

To further characterize the *Colletotrichum* isolates, pathogenicity assays were carried out in the greenhouses at the University of Kentucky (Lexington, KY) to determine their ability to infect sorghum and maize. Sweet sorghum lines 'Red Amber' and 'Sugar Drip', both highly susceptible to *C. sublineola* but resistant to *C. graminicola* (Xavier et al., 2018), and the maize inbred 'Mo17', which is highly susceptible to *C. graminicola* but resistant to *C. sublineola* (Buiate et al., 2017), were used. Seeds were planted in a 3:2 mixture of Pro-Mix BX (Premiere Horticulture, Ltd.) and sterile topsoil in Ray Leach Cone-tainers. One pellet of Osmocote Plus 15–9–12 was added to each of the sorghum cones at the time of planting. All cones were fertilized with Peters 20–10–20 at the rate of 0.15 g L^{-1} via Hozon Siphon Mixer on a weekly basis once the second true leaf had emerged from the whorl.

Fungal strains were cultured on PDA under continuous fluorescent light at 23 ˚C for 20 d. Spores were harvested from the plates, washed twice, and the concentration was adjusted to 5×10^5 spores ml^{-1}. One drop of Tween 20 (Sigma Aldrich) was added per 50 ml of spore suspension. When the sorghum plants had reached the V4–V5 stage (https://www.sorghumcheckoff.com/for-farmers/grain-production/growth-and-development), and the maize plants were at the V2 stage (https://www.agronomy.k-state.edu/extension/crop-production/corn/corn-growth-and-development.html), all plants were inoculated with the fungal strains via Chromist sprayer using a concentration of 5×10^5 spores ml^{-1}. Each strain was used to treat 10 plants of each of the plant lines, Red Amber, Sugar Drip, and Mo17. Known virulent strains of *C. sublineola* (CgSl1) and *C. graminicola* (M1.001)

TABLE 1 Species identification using the Basic Local Alignment Search Tool (BLAST) and expect values (*E* values) for internal transcribed spacer (ITS) sequence comparisons of fungal isolates collected in 2016 from several field locations in Pennsylvania

Isolate	Field location	Querylength	Putative *Colletotrichum* species	GenBank accession no.	Coverage, percent identity, *E* value	Pathogenicity	
						Sorghum	Maize
16ST1	Rock Springs	463	*truncatum*	MT704338	100%, 100% (0.0)	negative	negative
16ST2	Rock Springs	463	*truncatum*	MT704339	100%, 99.78% (0.0)	negative	negative
16ST3	Rock Springs	463	*truncatum*	MT704340	100%, 100% (0.0)	negative	negative
MJ2	Mount Joy	463	*truncatum*	MT704341	100%, 100% (0.0)	negative	negative
PSU1	Rock Springs	553	*graminicola*	MT704330	100%, 100% (0.0)	negative	positive
PSU2	Rock Springs	553	*graminicola*	MT704331	100%, 100% (0.0)	negative	positive
PSU3	Rock Springs	553	*graminicola*	MT704332	100%, 100% (0.0)	negative	positive
PSU4	Rock Springs	553	*graminicola*	MT704333	100%, 100% (0.0)	negative	positive
PSU5	Rock Springs	553	*graminicola*	MT704334	100%, 100% (0.0)	negative	positive
LB1	Lebanon	549	*sublineola*	MT704318	100%, 100% (0.0)	positive[a]	negative
LB3	Lebanon	549	*sublineola*	MT704319	100%, 100% (0.0)	positive[a]	negative
LB5	Lebanon	549	*sublineola*	MT704320	100%, 100% (0.0)	positive[a]	negative
LB6	Lebanon	549	*sublineola*	MT704321	100%, 99.82% (0.0)	positive[a]	negative
LB7	Lebanon	549	*sublineola*	MT704322	100%, 100% (0.0)	positive[a]	negative
PC2	Pillow	549	*sublineola*	MT704323	100%, 100% (0.0)	nt	nt
PC3	Pillow	549	*sublineola*	MT704324	100%, 100% (0.0)	nt	nt
PC4	Pillow	549	*sublineola*	MT704325	100%, 100% (0.0)	positive[b]	negative
PC5	Pillow	549	*sublineola*	MT704326	100%, 100% (0.0)	nt	nt
PC6	Pillow	549	*sublineola*	MT704327	100%, 100% (0.0)	nt	nt
PC7	Pillow	549	*sublineola*	MT704328	100%, 100% (0.0)	nt	nt
SL1	State Line	549	*sublineola*	MT704329	100%, 99.82% (0.0)	positive[c]	negative
PC8	Pillow	527	*spaethianum*	MT704335	100%, 99.62% (0.0)	positive[b]	negative
PC9A	Pillow	444	*spaethianum*	MT704336	100%, 99.55% (0.0)	positive[b]	negative
PC10A	Pillow	527	*spaethianum*	MT704337	100%, 99.43% (0.0)	positive[b]	negative

Note. nt, not tested.

[a]Only on Red Amber, not Sugar Drip.

[b]Low incidence in both Red Amber and Sugar Drip.

[c]High incidence in both Red Amber and Sugar Drip.

(Buiate et al., 2017), were used as positive controls. Negative controls were mock-inoculated with water. Cones were arranged in a completely randomized design (CRD) in cone racks, which were placed overnight in sealed black plastic bags to maintain high humidity. The next day, plants were removed from the plastic bags and placed on the greenhouse bench.

At 14 d post inoculation (dpi), plants were again placed in black plastic bags under high humidity for 24 h, before examining them under the microscope for the presence of setose acervuli characteristic of *Colletotrichum*. Only plants that developed acervuli were considered susceptible. Disease was measured as incidence: the number of plants that developed acervuli out of 10 that were inoculated per strain. The inoculation experiment, based on a method developed by Pande et al. (1994), was repeated twice.

2.2 | Disease response of sorghum in field and greenhouse assays

2.2.1 | Location of field and greenhouse experiments

Field experiments were conducted in central Pennsylvania at the Russel E. Larson Agricultural Research Station (Penn State University, Rock Springs, PA) during 2013, 2014, and 2016 in dedicated fields that had been planted the previous year with maize. Greenhouse trials to test the disease response of sorghum lines were carried out at the Department of Plant Sciences greenhouses at Penn State University during January–April 2016. Greenhouse conditions simulated

summer in Pennsylvania, i.e., 16 h light and an average temperature of 26 ˚C.

2.2.2 | Plant material

A total of 158 sorghum germplasm accessions were obtained from several sources including the USDA-ARS, Griffin, GA (25 lines); the Grain, Forage & Bioenergy Research, USDA-ARS, Lincoln, NE (56 lines); USDA-ARS, Lubbock, TX (10 lines); Texas A&M Agrilife Sorghum Breeding Program, College Station, TX (9 lines); National Plant Germplasm System (11 lines); Penn State University (17 lines); and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Pantancheru, India, (30 lines). The ICRISAT lines were chosen based on results of screening of 500 lines for ALB resistance under local conditions of Pantancheru, India. These ICRISAT screenings relied on natural infection by local foliar pathogens (Rao & Kumar, 2012). Thirty of the most resistant lines (disease score <2.0) were sent to Penn State University for further studies. Initial efforts were to study their performance in Pennsylvania, as described below. Partially resistant and susceptible lines (PAR3, PAW1, and PAW4) that were previously developed in our laboratory to study the role of flavonoids in ALB resistance were also included to compare their responses with the other sorghum lines (Ibraheem, Gaffoor, & Chopra, 2010). Sorghum lines H112 (susceptible to ALB) and IS19153 (resistant to ALB) were included as controls. Maize inbreds Mo940 (susceptible to ALB) and B73 (moderately resistant to ALB) were also included in the control group in an initial assessment experiment in the field.

2.2.3 | Initial assessment of sorghum performance in central Pennsylvania

Two fields at the Russell E. Larson Agricultural Research Center were used in 2013 and 2014 for uninoculated field trials involving 158 sorghum lines. In each case, the field had been planted the previous year with a commercial maize hybrid. All plantings were under a no-till regimen, and the maize residue was left in the field after harvest. The maize leaves and stubble were scouted for the symptoms or signs of *Colletotrichum* during both years.

The fields were initially treated with glyphosate (Roundup, Monsanto) 2 wk prior to planting for complete weed control. Seeds were treated with ConcepIII (Syngenta) at the rate of 0.4 g Fluxofenin kg^{-1} seed prior to sowing. Fertilizer (11–50–0) was applied at a rate of 111 kg ha^{-1} at the time of planting and the field was subsequently treated with the herbicide Lexar EZ (Syngenta) at the rate of 6 L ha^{-1}.

Each of the 158 entry plots consisted of two rows, each row 3.4 m in length, with 80-cm spacing between rows. The rate of sowing was 40–80 seeds row^{-1} depending on previous germination tests. Rows were thinned to 15 plants row^{-1} at the four-leaf stage.

The 158 sorghum accessions were subdivided into groups based on their plant height: dwarf (<1.5 m, 71 accessions); short (between 1.5 and 3 m, 67 accessions); or tall (>3 m, 20 accessions). Each group was then distributed in a randomized complete block design (RCBD) with three replications for each height category. Controls in these experiments were maize inbred B73 and Mo940 and sorghum lines H112 and IS19153. In addition, each tier contained a randomly placed plot of the susceptible check H112 to promote natural infestation of *Colletotrichum* spp. (Supplemental Table S1). Plants were scored for foliar disease symptoms 110 d post-planting by using a visual progressive scale (see trait assessment). All accessions were also evaluated for days-to-flowering and plant height (see 2.2.4 Trait assessment).

2.2.4 | Trait assessment

For field and greenhouse experiments, foliar symptom disease severity (hereafter referred to as "disease severity") was recorded on the third and fourth leaves below the flag leaf. Evaluation was done by use of a modified, visual progressive 1–9 scale, based on the proportion of the leaf that was symptomatic (Thakur, Reddy, & Mathur, 2007). A score of 3 or lower indicated a resistant response, whereas scores of 4–5 were considered moderately resistant and those greater than 5 were considered to be susceptible. The scale was: 1 = 0 to <1% leaf area covered with hypersensitive reaction of colored flecks; 2 = 1–5% leaf area covered with hypersensitive reaction of colored flecks; 3 = 6–10% leaf area covered with hypersensitive reaction of colored flecks; 4 = leaf area covered with yellow flecks and restricted necrotic lesions; 5 = 21–30% leaf area covered with yellow flecks and restricted necrotic lesions; 6 = 31–40% leaf area covered with coalescing necrotic lesions; 7 = 41–50% leaf area covered with coalescing necrotic lesions; 8 = 51–75% leaf area covered with coalescing necrotic lesions; and 9 = 76–100% leaf area covered with coalescing necrotic lesions. The color of the hypersensitive flecks is determined by the plant genotype and ranges from tan to red to a very deep purple (Ferreira & Warren, 1982). When screening for response to infection with *C. sublineola*, scores of 4–9 were assigned on the basis of the presence of acervuli within either the restricted or coalescing necrotic lesions.

Plants were also evaluated for days-to-flowering and plant height. Days-to-flowering was assessed as the number of days from planting to the time when 50% of the plants had florets

in the central part of the panicle at anthesis (de Almeida et al., 2014). Plant height was estimated by measuring the modal height from the ground to the tip of the fully emerged panicle in each entry plot.

2.2.5 | Assessment of reaction to *C. sublineola* in a subset of sorghum lines

A subset of the 158 sorghum lines consisting of 35 accessions, including the susceptible (H112) and resistant (IS19153) checks, was screened for anthracnose resistance. These accessions had low levels of foliar symptoms in the 2013 and 2014 field experiments, and included representatives of different sorghum types (forage, sweet, grain, forage/sweet). This experiment was conducted during the summer of 2016 in the greenhouse in the Department of Plant Science greenhouses at Penn State University as an RCBD with four replicates; each replicate consisted of four plants grown in 15-L pots containing field topsoil. Plants were grown to the V8 stage and inoculated with the known virulent strain CgSl1 of *C. sublineola* (originally from Indiana; Buiate et al., 2017) and only used in the greenhouse screening. The strain CgS11 was grown in OA medium for 10 d; spores were collected and adjusted to 1×10^6 prior to inoculations. Plants were spray-inoculated and placed in a high humidity chamber at 26 °C with natural lighting for 72 h to strongly favor disease development. Potted plants were subsequently returned to the greenhouse for the remainder of the experiment. Plants were scored 12 dpi using a visual, progressive 1–9 scale, as described above (see trait assessment; Pande et al., 1994; Thakur et al., 2007).

2.2.6 | Assessment of reaction to *C. sublineola* in a subset of sorghum lines in the field

The same subset of 35 accessions was inoculated with *Colletotrichum* in the field during the 2016 season. The experiment was set as an RCBD with four replicates. Inoculum was prepared by culturing a mixture of three pathogenic *Colletotrichum* strains isolated from fields in Pennsylvania (LB1, SL1, and PC10A; see Table 1) on autoclaved sorghum grain (Prom et al., 2009). Plants at approximately V8 stage were inoculated by placing a few of the *Colletotrichum*-infested sorghum seeds into the whorl. Irrigation by 1.8 m (6 foot) tall sprinklers in the early morning and late evening for 30 min each, for 3 wk post-inoculation, was carried out to maintain high humidity. Plants were scored for disease symptoms 45 dpi by using a visual progressive scale as described above (see trait assessment).

2.3 | Statistical analyses

Greenhouse pathogenicity assays conducted on sorghum lines Red Amber and Sugar Drip and maize inbred Mo17 were analyzed individually as a completely randomized design (CRD). The disease severity was analyzed via a non-parametric method that uses the ranked means (Brunner, Domhof, & Langer, 2002; Shah & Madden, 2004). Orthogonal contrasts between isolates and controls (water and isolate M1.001) were computed to determine differences in pathogenicity.

The field experiments conducted in 2013 and 2014 with 158 sorghum lines were analyzed as a RCBD with three replications. The disease severity data were subjected to a non-parametric analysis of the ranked means because of the use of a subjective marginal scale for the disease evaluation (Brunner et al., 2002; Shah & Madden, 2004) The analysis of variance was conducted using PROC MIXED of SAS Institute, combining the two experiments, as a nonsignificant genotype × environment interaction was detected. Replicates were nested within the experiments and the mean square of lines was used to obtain the $Pr > F$(DFF) value.

Relative marginal effects (RME) were calculated using the macro LD_CI (Brunner et al., 2002; Shah & Madden, 2004), calculated as follows:

$$\mathrm{RME} = (R - 0.5) / N$$

where R is the mean treatment ranking and N is the total number of experimental units for lines. Additional RME were calculated using as a classification sorghum height (dwarf, short, and tall) and sorghum types (sweet, grain, forage, and sweet/forage). Confidence Intervals (CI) of the RME were calculated at the 95% probability level (Brunner et al., 2002; Shah & Madden, 2004).

The experiments with 35 lines in the greenhouse and field were analyzed in a similar fashion as above using the nonparametric statistics described previously (Brunner et al., 2002; Shah & Madden, 2004). However, each experiment was analyzed individually. Linear contrasts were calculated to compare the differences between the newly tested lines and the lines considered susceptible (H112) and resistant (IS19153). In addition, linear contrasts were obtained among all accessions.

3 | RESULTS

3.1 | Collection and identification of Pennsylvania *Colletotrichum* isolates

Extensive surveys of stubble and debris in various fields across the state that were continuously cultivated with

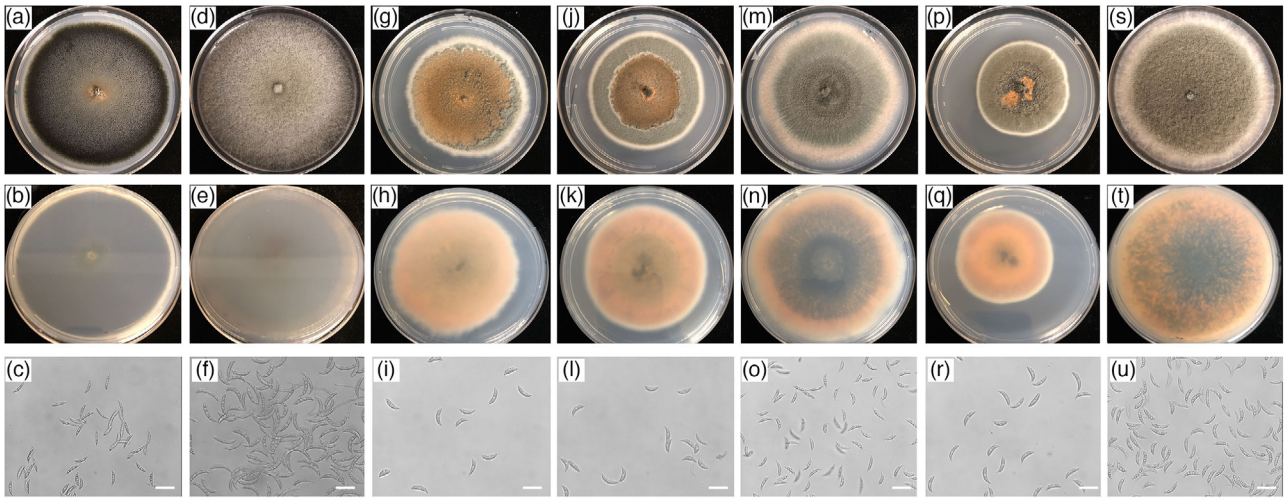

FIGURE 2 Appearance of representative *Colletotrichum* strains recovered from sorghum in Pennsylvania after 10 d of growth (dpi) on potato dextrose agar (PDA) under continuous fluorescent light. Each culture plate was photographed from the top (top row) and bottom (middle row). Spores (bottom row) were collected from the culture plates at 14 dpi, rinsed in water, and photographed on a Zeiss Axiscop with DIC optics at 400X. The scale bar represents 25 microns. a–c = 16ST1 (other cultures from this location and MJ2 from Mount Joy were similar morphologically to this one). d–f = PSU3 (all other cultures from this location were similar morphologically to this one). g–l = Two representative isolates from the Lebanon location: g–i = LB2; j–l = LB6. m–r = Two representative isolates from the Pillow location: m–o = PC9A; p–r = PC4. s–u = SL1 isolate from the State Line location

sorghum for at least 2 yr did not yield any *Colletotrichum* isolates (Figure 1). Numerous strains of a fungus that produced acervuli with setae but had spores with two polar, hyaline appendages, were isolated from these tissues (Supplemental Figure S1a). The ITS sequences suggested that these strains probably belonged to the genus *Minimidochium* (Supplemental Figure S1b). They did not cause any symptoms in the greenhouse assays on either sorghum or maize. Subsequent isolations in 2016 from symptomatic tissue on older sorghum leaves yielded several putative *Colletotrichum* strains (Table 1). Lesions typical of anthracnose were never observed on the younger, upper leaves of these plants. The ITS sequences of three isolates (16ST1, 16ST2, and 16ST3) from the Rock Springs, PA, location, and one from Mount Joy, PA (MJ2), matched *Colletotrichum truncatum* (Figure 2a–c, Table 1, Supplemental Figure S2). These isolates were not pathogenic to either sorghum or maize in the greenhouse assays (Table 2). The ITS sequences of five other isolates (PSU1, PSU 2, PSU3, PSU4, and PSU5) from Rock Springs, PA, confirmed that they belonged to *C. graminicola* (Figure 2d–f, Table 1, Supplemental Figure S2). These isolates were not pathogenic to sorghum but sporulated heavily on Mo17 maize (Table 2). The ITS sequences of five isolates from Lebanon (Figure 2g–l), six from the Pillow location (Figure 2p–r), and one from State Line, PA (Figure 2s–u), matched *C. sublineola* (Table 1, Supplemental Figure S2). These isolates were pathogenic to the Red Amber line of sorghum (Supplemental Figure S3a–d, Table 2). The SL1 isolate was also pathogenic to Sugar Drip. None of these isolates was pathogenic to maize. The ITS sequences of three other isolates from the Pillow site (PC8, PC9A, PC10A) matched *Colletotrichum spaethianum* and other members of the *C. spaethianum* complex (Figure 2m–o, Supplemental Figure S2). These isolates also produced sporulating lesions on Red Amber sorghum in greenhouse assays but were not pathogenic to maize (Table 2). With the exception of isolate SL1, all of the isolates from Pennsylvania that were pathogenic to sorghum produced relatively few sporulating lesions and were noticeably less aggressive than the CgSl1 positive control.

3.2 | Disease response of sorghum in field and greenhouse assays

Two field experiments were conducted in 2013 and 2014 involving 158 lines generated by breeding programs in the United States and at ICRISAT in India. There was significant genetic variability for disease severity among the lines ($P < .0001$); variation attributable to experiments as well as that to genotype × experiment interaction ($P > .05$) was not significant (Supplemental Table S1). The lines were classified by height as dwarf, short, and tall (see Materials and Methods). The RME of the disease severity on sorghum lines by height are shown in Figure 3. Dwarf lines had lower RME compared with short and tall lines overall.

The same lines were classified according to sorghum types. Sweet sorghum had the lowest RME, followed by grain,

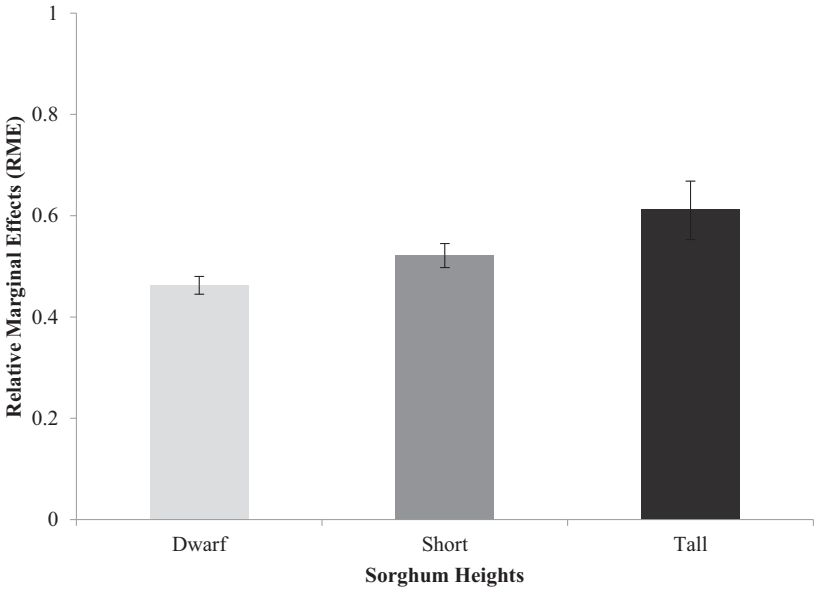

FIGURE 3 The relative marginal effects (RME) of the disease severity of sorghum genotypes classified according to plant height. The values come from combining data from two experiments conducted in Pennsylvania in 2013 and 2014. Error bars represent 95% confidence intervals

TABLE 2 Average disease incidence (DI) on sweet sorghum lines Sugar Drip and Red Amber and maize inbred Mo17 inoculated with putative isolates of *Colletotrichum* spp. collected in Pennsylvania in 2016

Isolate	Sugar Drip DI	Red Amber DI	Mo17 DI
Water	0	0	0[b]
16ST1	0	0	0[b]
16ST2	0	0	0[b]
16ST3	0	0	0[b]
MJ2	0	0	0[b]
PC8	0	0	0[b]
M1.001	0	0	100[c]
PSU1	0	0	100[c]
PSU3	0	0	100[c]
PSU4	0	0	100[c]
PSU5	0	0	100[c]
PSU6	0	0	100[c]
PC10A	0	50	0[b]
LB1	0	100[a]	0[b]
LB5	0	100[a]	0[b]
LB3	0	100[a]	0[b]
LB6	0	100[a]	0[b]
LB7	0	100[a]	0[b]
PC4	0	100[a]	0[b]
PC9A	5[a]	100[a]	0[b]
CgSL1	100[a]	100[a]	0[b]
SL1	100[a]	100[a]	0[b]

[a]Significantly different from isolate control M1.001 and water.
[b]Significantly different from isolate control M1.001.
[c]Significantly different from water.

forage, and sweet/forage (Figure 4). Seven of the 25 sweet sorghum lines had no lesions in the field experiments. One of these lines (B N110) was received from the Texas A&M Agrilife sorghum breeding program, whereas other lines had been developed for syrup production under growing conditions of high heat and high humidity (Bitzer, 1994) (Supplemental Table S2). In these experiments, 40 of the 95 lines of grain sorghum developed few or no foliar lesions; these lines originated from ICRISAT, and from breeding programs from Nebraska, Texas, Pennsylvania, and from the sorghum nested association mapping (NAM) panel (Supplemental Table S2). Eight lines classified as forage sorghum showed few to no lesions under field conditions; these lines were originally bred at ICRISAT and found to be resistant to ALB when tested at ICRISAT (Rao, Kumar, & Reddy, 2013; Rao, Blümmel, & Reddy, 2012; Thakur et al., 2010). In addition, five forage and three sweet/forage dual-use commercial hybrids also performed well when grown in central Pennsylvania. Lines FS5 (Monsanto) and SugarT (Advanta Seeds) were the best performers (Supplemental Table S2)

3.3 | Assessment of reaction to *C. sublineola* in a subset of sorghum lines

In these experiments, the response of a subset of 35 lines was evaluated after inoculation with *C. sublineola* in the greenhouse and under field conditions. Significant differences in disease severity were found among the sorghum lines infected with *C. sublineola* both in the greenhouse ($P < .0001$) and the field ($P < .0001$) (Supplemental Table S3). Overall, disease severity levels in the greenhouse were lower than those in the field (Table 3), but differences were only observed between the two genotypes (Supplemental Table S4).

TABLE 3 The disease severity (1–9) median, mean range, minimum, and maximum of sorghum genotypes evaluated in Pennsylvania in greenhouse conditions and field conditions in 2016

Genotype	Origin	Greenhouse					*P* value		Field					*P* value	
		Median	Mean	Range	Min.	Max.	H112[a]	IS19153[b]	Median	Mean	Range	Min.	Max.	H112	IS9513
IS19153	Sudan	4	3.8	7	1	8	.156	–	3	3.3	6	1	7	<.0001	–
H112	Indiana USA	5	4.7	8	1	9	–	.1560	9	8.5	5	4	9	–	<.0001
Ajabsido	Sudan	3	2.4	3	1	4	.0002	.0128	9	7.5	5	4	9	<.0001	<.0001
BTx623	Texas, USA	3	3.0	6	1	7	.0059	.2051	7	6.3	6	3	9	<.0001	<.0001
ICSB19	ICRISAT	1	1.5	3	1	4	<.0001	<.0001	5	4.6	8	1	9	<.0001	.0238
ICSB20	ICRISAT	5	4.8	6	2	8	.3569	.0338	6	5.7	7	1	8	<.0001	<.0001
ICSB389	ICRISAT	2	2.7	6	1	7	.0049	.0728	2	2.5	8	1	9	<.0001	.0034
ICSB430	ICRISAT	2	2.5	4	1	5	.0003	.0205	1	1.6	4	1	5	<.0001	.0010
ICSB431	ICRISAT	2	2.6	4	1	5	.0027	.0660	2	2.9	8	1	9	<.0001	.5286
ICSB474	ICRISAT	3	2.9	3	2	5	.0033	.2049	4	4.0	5	2	7	.0005	.3556
ICSB479	ICRISAT	3	2.9	5	1	6	.0085	.1474	2	2.5	6	1	7	<.0001	.0541
ICSB94	ICRISAT	1	1.9	5	1	6	<.0001	.0014	2	2.2	4	1	5	<.0001	<.0001
Macia	ICRISAT	1	2.0	5	1	6	<.0001	.0011	2	3.0	6	1	7	<.0001	.2891
N321	Nebraska USA	4	3.8	4	1	5	.3866	.5730	6	5.7	5	4	9	<.0001	<.0001
N322	Nebraska USA	2	2.9	6	1	7	.0052	.1158	4	4.1	5	2	7	<.0001	.1251
N327	Nebraska USA	4	4.3	8	1	9	.6022	.6183	4	4.1	6	2	8	<.0001	.0251
N329	Nebraska USA	2	1.9	2	1	3	<.0001	<.0001	7	6.3	8	1	9	<.0001	<.0001
N330	Nebraska USA	4	4.3	7	1	8	.8290	.2593	6	5.8	4	4	8	<.0001	<.0001
N332	Nebraska USA	2	2.6	4	1	5	.0008	.0584	5	5.2	6	2	8	<.0001	<.0001
N334	Nebraska USA	2	2.6	5	1	6	.0019	.0505	4	4.3	6	2	8	<.0001	.0021
N338	Nebraska USA	4	3.3	5	1	6	.0870	.6099	7	6.7	6	3	9	<.0001	<.0001
N340	Nebraska USA	6	5.8	6	2	8	.0303	.0008	6	6.3	7	2	9	<.0001	<.0001
P898012	Indiana USA	4	4.4	6	2	8	.8652	.1532	6	5.7	6	3	9	<.0001	<.0001
SC1103	Nigeria	2	2.5	7	1	8	.0008	.0260	9	7.9	5	4	9	.0014	<.0001
SC1345	Mali	2	2.3	5	1	6	<.0001	.0041	3	4.1	7	2	9	<.0001	.0834
SC265	Burkina Faso	2	2.7	3	1	4	.0010	.0600	2	2.4	2	2	4	<.0001	.0002
SC283	Tanzania	2	2.1	5	1	6	.0001	.0050	3	3.0	3	2	5	<.0001	.1020
SC35	Ethiopia	1	1.8	3	1	4	<.0001	.0003	5	4.9	5	3	8	<.0001	<.0001
SC971	Puerto Rico	2	2.9	7	1	8	.0041	.0890	7	7.2	7	2	9	<.0001	<.0001
Segaolane	Botswana	2	2.3	5	1	6	.0004	.0126	7	6.1	7	2	9	.0150	.0084
Simon	unknown	4	3.9	5	2	7	.4787	.5294	4	4.3	5	2	7	<.0001	.0010
Sugar Drip	unknown	5	5.2	7	2	9	.3539	.0259	4	4.1	4	3	7	<.0001	.0085
Tx430	Texas USA	2	2.3	4	1	5	.0002	.0096	4	4.5	6	2	8	<.0001	.0004
PAR3	Sudan	4	2.9	3	1	4	.0028	.1203	7	7.2	6	3	9	.0001	<.0001
PAW4	Sudan	4	3.9	6	2	8	.4619	.6224	7	7.0	4	5	9	.006	<.0001

[a]H112 is the susceptible control.
[b]IS19153 is the resistant control.

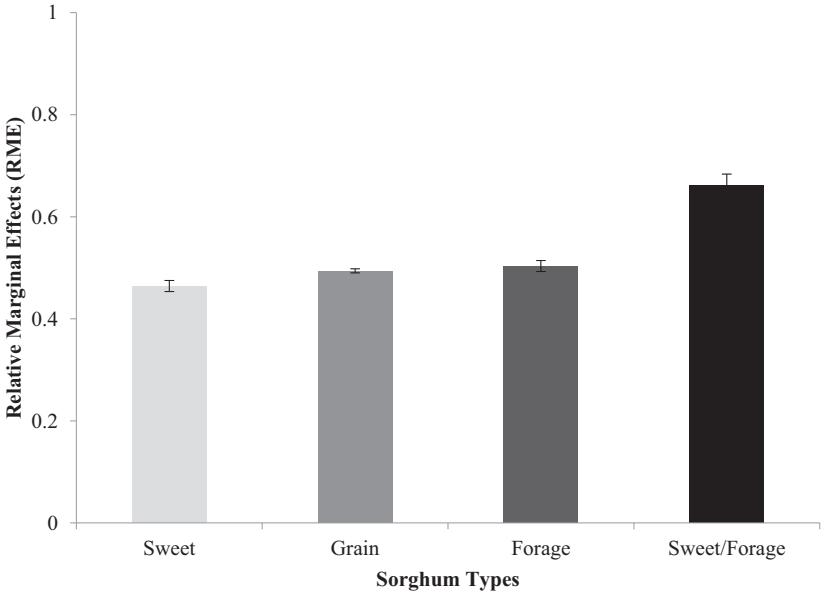

FIGURE 4 The relative marginal effects (RME) of the disease severity of sorghum genotypes classified according to types. The values come from the combination of two experiments conducted in Pennsylvania in 2013 and 2014. Error bars represent 95% confidence intervals

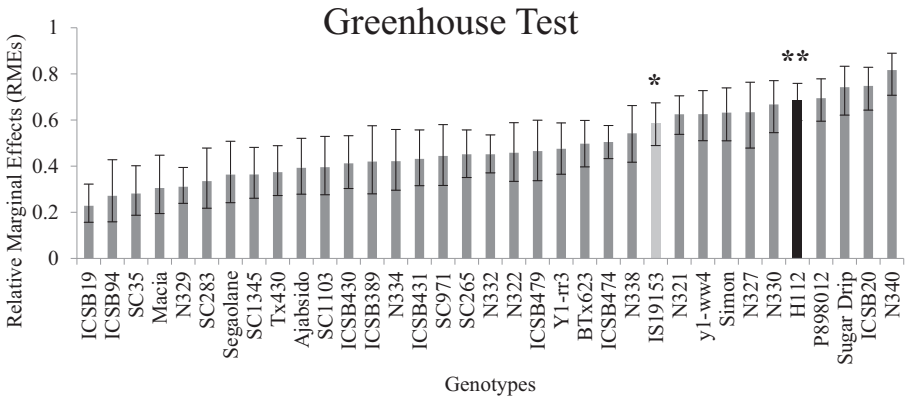

FIGURE 5 Relative marginal effects of the disease severity and their confidence intervals (95%) of 35 sorghum genotypes evaluated in greenhouse conditions in 2016 inoculated with *C. sublineola* isolate CgSl1 in Pennsylvania. The resistant (IS19153, *) and susceptible (H112, **) controls are indicated by asterisks. Error bars represent 95% confidence intervals

In the greenhouse, the lines with low RME were ICSB19 (0.23), ICSB94 (0.27), SC35 (0.28), and Macia (0.30) (Figure 5). These lines had a median value of 1, indicating that these lines had no disease symptoms. The susceptible control line H112, with an RME of 0.69, reached a median value of 5 on the rating scale (21–30% leaf area covered with yellow flecks and restricted necrotic lesions). However, this line showed milder symptoms in the greenhouse when inoculated with the virulent CgSl1 strain in comparison with field tests. In this experiment, 22 lines were significantly more resistant than the susceptible control (H112) (Figure 5); four of those lines were also significantly more resistant than the resistant control (IS19153), according to the *P* values of the linear contrasts (Table 3).

In the field, the RMEs of ICSB430 (0.10), ICSB94 (0.16), SC265 (0.18), and ICSB389 (0.19) were the lowest among the 35 evaluated lines. These four lines were significantly more resistant than the resistant control line IS19153 (0.30); the resistant control had a median disease severity value of 3. H112, the susceptible control had a disease severity value of 9, and nearly all sorghum lines differed from the susceptible control H112 (0.89) according to their lineal contrasts (Figure 6, Table 3).

Comparing all three experiments (i.e., field/uninoculated, field/inoculated with a mix of local *C. sublineola* isolates, and greenhouse/inoculated with the virulent CgSl1 strain), the responses could be broadly classified into four categories. The categories were: (a) lines moderately resistant to foliar pathogens under all conditions (12); (b) lines susceptible under all conditions (9); (c) lines susceptible to *C. sublineola* in both the greenhouse and field (4); and (d) lines susceptible to the mix of *C. sublineola* strains in the field but not to CgSl1 in the greenhouse (10).

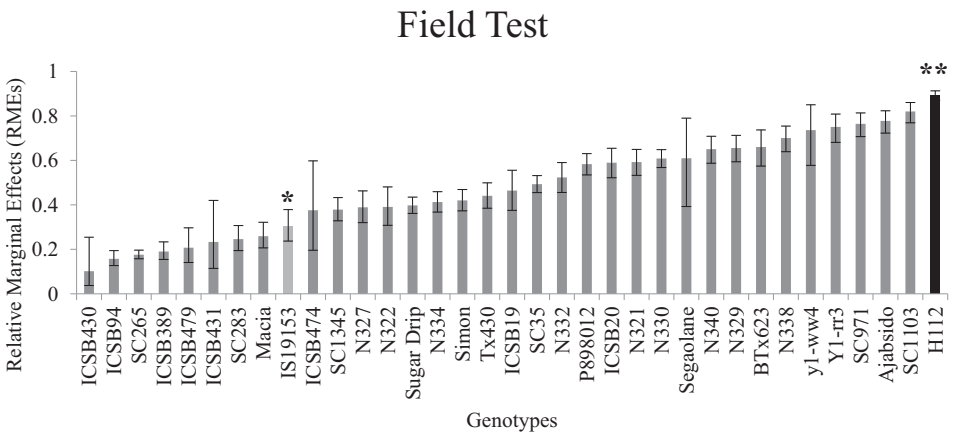

FIGURE 6 Relative marginal effects of the disease severity and their confidence intervals (95%) of 35 sorghum genotypes evaluated in field conditions in 2016 inoculated with *C. sublineola*, mix of strains LB1-7, SL1, and PC1-10. The resistant (IS19153, *) and susceptible (H112, **) controls are indicated by asterisks. Error bars represent 95% confidence intervals

4 | DISCUSSION

Colletotrichum sublineola is known to survive in crop debris. However, fields in Pennsylvania that had been under continuous sorghum cultivation for at least 2 yr did not yield any *Colletotrichum* isolates surviving on the stubble and debris, indicating that populations of this pathogen in these fields are still very small. A fungus apparently belonging to the genus *Minimidochium* was recovered. Members of this genus are reported to be saprophytic on leaf litter (Cabello, Arambarri, & Cazau, 1998; Lunghini, Granito, Di Lonardo, Maggi, & Persiani, 2013; Sutton, 1969). To our knowledge, this is the first report of this fungus in sorghum stubble, although it has been previously isolated from *Miscanthus* in Illinois and Louisiana (Shrestha et al., 2015). The superficial resemblance of its setose acervuli to *Colletotrichum* may result in misdiagnosis if only a hand lens is used for detection, and spores are not subsequently inspected.

Strains of *C. sublineola* were isolated from lesions that occurred at low incidence on older, senescing, lower leaves of healthy sorghum plants. Most of the *C. sublineola* isolates, with the exception of the one from State Line Pennsylvania, were not very aggressive in comparison with the control strain CgSl1 from Indiana, causing only relatively few sporulating lesions, even on the highly susceptible Sugar Drip and Red Amber sorghum lines. This low level of aggressiveness could explain why *C. sublineola* failed to cause disease symptoms on younger, upper leaves of the sorghum plants, and only limited numbers of lesions on the older leaves. Nonetheless, this study confirmed that the pathogen is present, and we can expect levels of inoculum to increase, and for the population to become more aggressive as a result of increased selection pressure, if the area under sorghum continues to increase in the state.

Colletotrichum graminicola and another species, likely *C. truncatum*, were also isolated from older senescing sorghum leaves at the Rock Springs, PA, location. The *C. graminicola* strains were pathogenic to maize but not to sorghum in the greenhouse assays. This finding demonstrates the potential for *C. graminicola* to be carried over in senescing sorghum tissues during rotations. *Colletotrichum truncatum* has been isolated from *S. halepense* in Mississippi (McLean & Roy, 1991), and in Puerto Rico (Lenné, 1990), but it has not been reported on sorghum. The putative *C. truncatum* strains were not pathogenic to healthy sorghum or maize in the greenhouse, suggesting that they are not highly adapted to the crop and were opportunistically colonizing the senescing lower leaves, like *C. graminicola*. One other species that was isolated from the Pillow, PA, location was identified to be *C. spaethianum* or a member of that species complex, based on ITS sequences. Members of this species complex are reportedly pathogens of various dicots, including soybean [*Glycine max* (L.) Merr.], but have not been found previously on sorghum or other monocots. Surprisingly, these strains were pathogenic to sorghum in the greenhouse assays, although the number of sporulating lesions produced was small. Nonetheless, because they were pathogenic, a representative was included with the *C. sublineola* isolates in the inoculum that was used for field assessment.

Initial field screenings involving exposure to ambient pathogen inoculum indicated genetic variation among 158 sorghum lines in the development of foliar disease symptoms. This is evinced in the range and diversity of symptoms observed—from lines with no or highly dispersed colored flecks to the other extreme of the scoring scale where a large portion of the leaf was covered in coalescing, necrotic lesions and all categories between. The lines were classified on the basis of height because plant height and days to flowering are known to be important contributors to agro-climatic

adaptation and to the expression of phenotypic traits such as days to seed maturity, seed weight,and so forth (Bouchet et al., 2017; Buckler et al., 2009; Russell et al., 2016). Dwarf sorghum had, by far, the least foliar disease symptoms. This group consisted of converted grain sorghum lines comprising alien germplasm with desirable agronomic traits, such as resistance to diseases and insects, which had adapted to climatic conditions in the United States (Stephens, Miller, & Rosenow, 1967). When classified according to usage, sweet sorghums performed the best overall with respect to reduced foliar lesions in this study. The sorghum lines, such as Della, Dale, and Sugardrip, were bred for ALB resistance in regions of the United States, such as Virginia and Mississippi, where disease pressure is relatively high (Bitzer, 1994). Furthermore, these lines were primarily tall (1.5–3.7 m when grown in Pennsylvania). We hypothesized that these lines might have escaped secondary rounds of infections from inoculum produced on lower leaves or crop debris. In contrast, early maturity and short-stature lines, such as BSD106, N330, Tx475, were associated with greater susceptibility to foliar pathogens than the late-maturing, tall-stature lines (Wang et al., 2014).

Lines adapted to warmer regions are day-length sensitive and require short days to flower. Lines ICSB221, ICSB211, and ICSB25002 flowered very late in the season and did not produce mature seeds in central Pennsylvania (Stephens et al., 1967). In fact, two lines (X 98456 and TAMUXH08001) did not flower prior to frost damage at the end of the growing season, which confirmed the lack of adaptation of this group of sorghum lines to Pennsylvania environmental conditions. Lines from ICRISAT were selected for resistance to *C. sublineola* in India (Thakur et al., 2010) and our results indicate that this resistance continued to be expressed under conditions prevalent in central Pennsylvania. ICRISAT lines ICSB389, ICSB430, ICSB431, ICSB474, ICSB479, ICSB94; NAM parental lines Macia, SC1345, SC265, SC283, Tx430; and the resistant check IS19153, showed few to no symptoms in all three trials. Line Tx430 has been found to be highly susceptible to ALB in experiments conducted in climates more conducive to the disease, such as Puerto Rico, Georgia, and Texas, and with different, possibly more aggressive isolates of *C. sublineola* (Cuevas, Prom, & Cruet-Burgos, 2019; Prom, Ahn, Isakeit, & Magill, 2019). All these lines flowered between 83 and 99 days and had height varying from 0.9 to 2.3 m. They were mostly classified as forage and grain sorghum and would likely be well adapted to the Pennsylvania summer environment. Perhaps they were more resistant because they did not flower. Plants in general are more susceptible to biotic stresses during the reproductive phase, because of the energy expenditure involved (Ayres et al., 1996). The cooler temperatures with the onset of fall results in reduced disease pressure when plants are most vulnerable (Erpelding, 2008). This, in turn, would result in less disease in the late-maturing/flowering lines. Increased foliar growth attributable to the lack of or delayed flowering make lines TAMUXH08001, ICSB221, and ICSB211 ideal candidates for biomass sorghum.

Lines ICSB20, N338, Sugar Drip, and Simon, which had low levels of disease in the initial uninoculated trial, were highly susceptible when inoculated with *C. sublineola* in the greenhouse or in the field. One hypothesis is that foliar symptoms were caused by pathogens other than *C. sublineola* in the uninoculated study. This hypothesis would be consistent with the low incidence of the pathogen during the initial surveys.

Some lines (Ajabsido, SC1103, SC35, SC971, Segaolane, ICSB19, BTx623, N329, N332, and N334) that were susceptible when inoculated with local isolates of *C. sublineola* in the field did not succumb to ALB under greenhouse conditions when inoculated with the more pathogenic CgSl1 strain. Previous studies have yielded similar results, in which field inoculations yielded more intense symptoms than greenhouse inoculations (Ferreira & Warren, 1982; Xavier et al., 2017). This could be attributed to the field plants being subjected to higher levels of biotic and abiotic stresses, including infection by many other pathogens, as opposed to greenhouse conditions, where sorghum was provided with water, nutrients, and temperature, which could have helped to build defense against the pathogen. Some of these lines, e.g., SC1103 and SC971 (Cuevas, Prom, & Cruet-Burgos, 2019), and SC35 and Segaolane (Prom, Ahn, Isakeit, & Magill, 2019) had been previously cataloged as resistant to ALB. Discrepancies between this and other studies, and between greenhouse and field in the current study might be attributed to differences in pathogen strains, or to differences in the environmental and experimental conditions. Additional pathogenicity tests should be conducted to elucidate the race structure of isolates of *Colletotrichum* collected in Pennsylvania.

Although numerous studies have been carried out in the United States to identify sources of resistance to ALB in exotic germplasm (Cuevas, Prom, Erpelding, & Brotons, 2014; Cuevas, Prom, Isakeit, & Radwan, 2016; Prom et al., 2011), few have focused on commercially grown hybrids. Similar to our study, Isakeit et al. (2008) found 6 of the 30 commercial hybrids that they tested to be resistant to ALB, indicating that genetic variation for resistance in sorghum against ALB exists in commercial hybrids (FS5 and SugarT). The most resistant commercial hybrids in this study, which were forage and sweet/forage hybrids, remained vegetative for most of the growing season. The parental inbred lines of these hybrids are not known. Therefore, we cannot recommend the best hybrid combinations. However, seed companies could cross the ALB resistant inbred lines to locally adapted inbred lines and determine their general and specific combining abilities for resistance to foliar diseases, including ALB, under Pennsylvania conditions, allowing them to make recommendations on the best hybrid combinations useful for environmental conditions prevalent in Pennsylvania.

Sorghum is believed to have originated in northeastern Africa, with a secondary center of origin in the Indian subcontinent (Hariprasanna & Patil, 2015). Lines developed by ICRISAT are diverse in pedigree (Supplemental Table S5) and are likely to have a wider genetic variability than other lines used in this study. They comprise landraces from India (IS 18432), Kenya (PS21303), Australia (IS 18757), Sudan (IS 18953, IS 9664, IS 9667), Malawi (IS 21599), and Tanzania (3436) that originated in the secondary center of origin of sorghum (Leppik, 1970; Zhang et al., 2014). Sorghum germplasm from the wet and humid regions of Africa have been selected under high levels of anthracnose disease pressure and therefore are an important source of resistance to fungal diseases, such as ALB (Cuevas et al., 2019; Erpelding, 2008). ICRISAT lines ICSB389, ICSB430, ICSB431, and ICSB94 were identified as moderately resistant against *C. sublineola* in our experiments both in the field and in the greenhouse. The line Macia, originally from Zimbabwe, was also found to be moderately resistant in all three trials in our study, but a similar study classified this accession as susceptible in Texas (Prom et al., 2019). Therefore, Macia may have less resistance to some North American isolates of *C. sublineola*.

5 | CONCLUSIONS

Strains of *C. sublineola* that cause ALB on sorghum were identified in several Pennsylvania locations, indicating the possibility of future outbreaks of ALB. Several species of *Colletotrichum*, including *C. graminicola*, were also identified on senescent sorghum tissue, demonstrating the potential for sorghum to serve as a reservoir for non-pathogens. This is the first report of *C. graminicola* colonizing sorghum in the field and shows the potential for exacerbating anthracnose outbreaks in both sorghum and maize when grown in rotation, as currently occurs in Pennsylvania. A higher resistance to foliar disease was found within sorghum lines classified as dwarf (e.g., B N122, B N128, B N32) and sweet (e.g., B N110, Bailey, Clubhead) compared to those classified as short, tall, grain, forage, and sweet/forage based on height and type. However, breeders can choose sources of resistance within sweet, grain, forage, or the forage/sweet types, as we evaluated a large diversity panel of sorghum lines. Most of the resistant lines bred at ICRISAT (classified as grain, sweet, and forage sorghum) had a wider spectrum of resistance to isolates of *C. sublineola* in the field and to one very pathogenic strain in the greenhouse compared with other lines used in this study. In this study, we demonstrated the value of ICRISAT (ICS) lines ICSB94, ICSB430, and ICSB431 as novel sources of moderate resistance suitable for incorporation into sub-tropical and temperate breeding materials to improve sorghum for ALB resistance in Pennsylvania and the northeastern United States.

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AUTHOR CONTRIBUTIONS

Iffa Gaffoor: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Writing-original draft; Writing-review & editing. Germán V. Sandoya: Investigation; Methodology; Validation. Etta M. Nuckles: Investigation; Validation. Srinivasa R. Pinnamaneni: Data curation; Investigation; Methodology; Supervision; Writing-review & editing. Lisa J. Vaillancourt: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Writing-original draft; Writing-review & editing. Surinder Chopra: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Writing-original draft; Writing-review & editing.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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