Pathotypes of *Colletotrichum sublineolum* in Arkansas

J. W. Moore, Former Senior Graduate Assistant, M. Dittmore, Research Associate, and D. O. TeBeest, University Professor, Department of Plant Pathology, University of Arkansas, Fayetteville 72701

ABSTRACT

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Sorghum anthracnose, caused by *Colletotrichum sublineolum*, is widely distributed in most grain sorghum-producing areas in Arkansas, and localized epidemics continue to occur across the state. The objectives of this research were to determine the number of pathotypes of *C. sublineolum* in Arkansas and to determine where these pathotypes are located within the state. Ninety-eight isolates were collected from research stations and production fields located in the Delta region of Arkansas from 2003 through 2005 and were tested for virulence to eight differential grain sorghum breeding lines. Of these, 11 isolates produced conflicting and unreliable results and were excluded from further analysis. Among the remaining 87 isolates, 13 pathotypes were identified. Eleven of these pathotypes were previously unreported, with 53% of the isolates consisting of pathotype 9. Pathotype 9 was distinguished from all other pathotypes by its virulence to all eight differential lines. Pathotype diversity appeared to be more closely associated with the year than with the locations where isolates were collected. Although all host differential lines were susceptible to at least 50% of the isolates evaluated, inbred lines SC326-6, TAM428, and TX2536 were susceptible to more than 90% of the 87 isolates evaluated. These results showed that the *C. sublineolum* populations in Arkansas are diverse based on the comparisons of pathogenicity to previously published host differentials, and it appears that these field populations varied from year to year.

Additional keywords: anthracnose severity, pathotype determination

Sorghum anthracnose, caused by *Colletotrichum sublineolum* (Henn.) in Kabat & Bubak (formerly *C. graminicola* (Ces.) G.W. Wilson), is an important disease in nearly all grain sorghum (*Sorghum bicolor*) production regions of the world. This disease infects and damages leaves, stalks, peduncles, and panicles (5,16,17) and causes estimated yield losses exceeding 50% on highly susceptible cultivars (6). Yield losses may be attributed to both reduced kernel number and size due to floret mortality, because Ngugi et al. (9) reported that sorghum anthracnose developed slowly before anthesis and increased rapidly during the grain-filling stage.

Before 1985, sorghum anthracnose was considered to be of minor importance in Arkansas; however, in 1985, *C. sublineolum* severely infected grain sorghum fields in southwest Arkansas, causing significant yield reductions (4). At that time, it was not known whether the outbreak was caused by abnormal environmental condi-

tions or whether the pathogen in this area had adapted and become more virulent than before (4). Since then, numerous studies have been conducted to evaluate the level of resistance in different sorghum hybrids to sorghum anthracnose (12).

It has been suggested that one of the most effective control strategies for sorghum anthracnose is disease resistance (13), and many of the inbred lines used in popular hybrids were developed for anthracnose resistance in areas of the southern United States (12). However, management of this disease through host resistance has often been unsuccessful because this pathogen is considered to be hypervariable (1,2,10). Additionally, several pathotypes and haplotypes of this fungus have been reported (1,2,4,11,14,15). Eight pathotypes of *C. sublineolum* have been reported among isolates collected in Georgia, Puerto Rico, and Texas. Pathotypes 7 and 8 were the most virulent on the sorghum differentials tested (2). Notably, pathotype 7 from Puerto Rico and pathotype 8 from Georgia were virulent on all sorghum differentials except SC414-12E and BTX378, respectively (2). Similar experiments using the same set of sorghum differentials compared an Arkansas isolate with an isolate from the Brazos River Bottoms of Texas (4). Results from these studies indicated that the Arkansas isolate caused more disease than the Texas isolate on breeding lines SC326-6 and BTX378 (4). In Georgia, nine *C. sublineolum* haplo-

types were identified in a field where several seed companies tested their breeding lines for anthracnose resistance (11).

Although anthracnose is widely distributed in Arkansas, information is lacking on the number of pathotypes and their distribution within the state. Without reliable information on pathotypes, recommendations to Arkansas producers on which grain sorghum hybrids to plant are difficult, if not impossible, to develop. Therefore, the objectives of this research were to determine (i) the number of pathotypes of *C. sublineolum* in Arkansas and (ii) where these pathotypes exist within the state.

MATERIALS AND METHODS

Isolate collection, isolation, and storage. In 2003, 2004, and 2005, 98 *C. sublineolum* isolates in all were collected from production fields located in the major grain sorghum production areas within the state and from cultivar test plots at the University of Arkansas Pine Tree Branch Station near Colt, AR and at the University of Arkansas Lon Mann Cotton Research Station near Marianna, AR. Information for each isolate regarding the city, county, year, and sorghum hybrid from which each isolate was collected can be found in Table 1. Because anthracnose develops in patches within a field, leaf samples with characteristic anthracnose lesions were collected randomly from these foci in the field at sorghum maturity, placed in a plant press, and transported to the University of Arkansas for pathogen isolation.

Small sections of individual lesions (0.5 cm²) were collected from each leaf and immediately placed in sterile water for 1 min followed by a 2-min soak in 1% sodium hypochlorite. After the lesions were disinfested, each was placed on Emerson yeast extract agar (YpSS) medium (4 g of yeast extract, 15 g of soluble starch, 1 g of K₂HPO₄, 0.5 g of MgSO₄, and 16 g of agar per liter), as described previously by Li (7), and placed in an incubator at 25°C under fluorescent lights (GE warm white brightstik 33 with a fluorescent light output of 725 lumens) with a 16-h photoperiod. Once these field cultures began to sporulate, a sterile loop was used to transfer spores into a 2-ml centrifuge tube containing 1 ml of distilled water in order to dilute the spores. Approximately 20 µl of the spore mixture was streaked onto 2% water agar medium (20 g of agar per liter of distilled water), then placed in an incubator at 25°C under fluorescent lights with a 16-h photoperiod to promote spore ger-

Corresponding author: D. O. TeBeest
E-mail: dtebeest@uark.edu

Current address of J. W. Moore: Senior Station Superintendent, Cimarron Valley Research Station, Oklahoma State University, Perkins 74059.

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mination. After 48 h, individual germinated spores were collected using a dissecting microscope and transferred to YpSS agar medium, then placed in an incubator at 25°C under fluorescent lights with a 16-h photoperiod to promote colony growth and sporulation.

After the single spore cultures started to sporulate, mycelium plus spores were harvested and placed into 2-ml sterile, cryovials (Wheaton, Millville, NJ) containing a cryopreservative solution (1:1 solution of 10% nonfat dry milk and 40% glycerol) for long-term storage. All single-spore cultures were stored in a cryofreezer (−80°C).

Host differentials. Selected grain sorghum inbred lines, previously used to iden-

tify pathotypes (2), were obtained from William L. Rooney, Texas A&M University. Grain sorghum seed were planted in Jiffy-7 peat pellets (Hummert International, Earth City, MO) at a rate of one seed per peat pellet. Following germination, seedlings were allowed to grow to the one- to two-leaf stage before being transplanted to 25-cm-diameter plastic pots filled with loam, peat, and sand (4:4:1, vol/vol/vol). Three sorghum seedlings were transplanted to each pot and placed in a greenhouse at 24 to 32°C, with a photoperiod of 16 h. In the absence of natural sunlight, quartz halogen lamps were used to supplement natural lighting for the duration of the experiment. All plants were fertilized with All Purpose

Plant Food (Miracle-Gro Products, Inc., Marysville, OH) at 14 and 28 days after planting (DAP).

Inoculum preparation and inoculation. Single-spore isolates were grown on YpSS agar medium at 25°C under fluorescent lights with a 16-h photoperiod to promote sporulation. Inoculum was prepared by flooding 10-day-old cultures with 20 ml of distilled water followed by filtering the conidial suspension through three layers of cheesecloth. The final spore concentration for each isolate was adjusted to approximately 1×10^6 spores ml^{−1} using a hemacytometer.

Approximately 40 to 45 DAP, the host differentials were inoculated with conidial

Table 1. Pathotype designation, origin, year of collection, and source hybrid for each of the *Colletotrichum sublineolum* isolates evaluated in this research

Isolate	Pathotype ^y	Origin		Year	Hybrid
		Location, AR ^z	County		
AR551	11	Forrest City	St. Francis	2003	Asgrow A571/Farmer's field
AR552	10	Forrest City	St. Francis	2003	Asgrow A571/Farmer's field
AR553	11	Forrest City	St. Francis	2003	Asgrow A571/Farmer's field
AR555	14	Forrest City	St. Francis	2003	Asgrow A571/Farmer's field
AR556	11	Forrest City	St. Francis	2003	Asgrow A571/Farmer's field
AR558	10	Marianna	Lee	2003	Dyna Gro X17F90
AR562	10	Marianna	Lee	2003	Dyna Gro X17F90
AR564	10	Marianna	Lee	2003	Dyna Gro X17F90
AR565	7	Marianna	Lee	2003	Terral TV97H17
AR568	9	Marianna	Lee	2003	Terral TV97H17
AR569	12	Marianna	Lee	2003	Terral TV97H17
AR571	9	Marianna	Lee	2003	DEKALB DKSS4-00
AR572	9	Marianna	Lee	2003	DEKALB DKSS4-00
AR573	8	Marianna	Lee	2003	DEKALB DKSS4-00
AR575	17	Marianna	Lee	2003	DEKALB DKSS4-00
AR576	9	Marianna	Lee	2003	Monsanto X234
AR577	8	Marianna	Lee	2003	Monsanto X234
AR579	13	Marianna	Lee	2003	Monsanto X234
AR580	8	Marianna	Lee	2003	Monsanto X234
AR581	11	Marianna	Lee	2003	Monsanto X234
AR583	7	Marianna	Lee	2003	FFR 318
AR587	9	Marianna	Lee	2003	FFR 318
AR588	8	Pine Tree	St. Francis	2003	Dyna Gro 732B
AR589	8	Pine Tree	St. Francis	2003	Dyna Gro 732B
AR590	8	Pine Tree	St. Francis	2003	Dyna Gro 732B
AR592	8	Pine Tree	St. Francis	2003	Dyna Gro 732B
AR594	7	Pine Tree	St. Francis	2003	Triumph TR461
AR595	7	Pine Tree	St. Francis	2003	Triumph TR461
AR597	9	Pine Tree	St. Francis	2003	Triumph TR461
AR599	9	Pine Tree	St. Francis	2003	Triumph TR461
AR600	7	Pine Tree	St. Francis	2003	Triumph TR461
AR601	7	Pine Tree	St. Francis	2003	Triumph TR459
AR603	9	Pine Tree	St. Francis	2003	Triumph TR459
AR605	9	Pine Tree	St. Francis	2003	Triumph TR459
AR606	9	Pine Tree	St. Francis	2003	Triumph TR459
AR607	10	Marianna	Lee	2003	Unknown/Field isolate
AR612	10	Marianna	Lee	2003	Dyna Gro X17F30
AR615	18	Marianna	Lee	2003	Unknown/Field isolate
AR623	9	Marianna	Lee	2004	Terral TV9421
AR625	9	Marianna	Lee	2004	Dyna Gro 780B
AR627	9	Marianna	Lee	2004	FFR 322
AR628	9	Marianna	Lee	2004	Terral TV96H81
AR634	9	Marianna	Lee	2004	Pioneer 84G62
AR635	9	Marianna	Lee	2004	Dyna Gro 780B
AR646	9	Marianna	Lee	2004	Dyna Gro 751B
AR647	9	Marianna	Lee	2004	Asgrow A571

(continued on next page)

^y Pathotype designation is based on the virulence pattern of each Arkansas isolate to a subset of inbred sorghum breeding lines SC326-6, SC414-12E, BTX378, TAM428, TX2536, SC328C, QL3 (India), and BTX398 as described by Cardwell et al. (2). Eleven isolates were designated as ? (= unknown) because different reactions were observed on some of the host differentials between repetitions.

^z All isolates collected at Marianna were isolated from plants grown on the Cotton Branch Research Station near Marianna, AR. Isolates collected at Pine Tree came from the Pine Tree Branch Research Station near Colt, AR. The remaining isolates were collected from producer fields located in the designated county.

suspensions from each isolate using an airbrush sprayer with compressed air. Immediately following the inoculation, the plants were placed in a dew chamber at 25°C for 24 h. After this 24-h period, the plants were removed from the dew chamber and returned to the same greenhouse for the duration of the experiment.

Pathotype determination. Sorghum differentials were evaluated for lesion type reactions at 14 days after inoculation (DAI) as described by Cardwell et al. (2) and Dale et al. (4) using the following scale: resistant (R) = very small lesions with no evidence of lesion expansion or increase in size; moderately resistant (MR) = lesions larger (1 to 3 mm), but not generally associated with sporulation or sig-

nificant chlorosis; moderately susceptible (MS) = lesions larger (4 to 6 mm) and associated with chlorosis and some sign of sporulation on a few lesions; and susceptible (S) = lesions generally large (>6 mm), with significant chlorosis and sporulation occurring on >50% of the lesions. All R and MR lesion-type reactions were designated as (–) and all MS and S reactions were designated as (+). These minuses (–) and pluses (+) were used to generate a virulence pattern for each isolate on all eight host differentials.

Anthracnose severity. In addition to the lesion-type reaction ratings, disease severity was determined for each sorghum host differential 14 DAI using a modified Horsfall-Barratt scale, where 0 = none, 1 = 0 to

3%, 2 = 4 to 6%, 3 = 7 to 12%, 4 = 13 to 25%, 5 = 26 to 50%, 6 = 51 to 75%, 7 = 76 to 88%, 8 = 89 to 94%, 9 = 95 to 97%, and 10 = 98 to 100%. Ratings were converted to the midpoint percentages before analysis.

Experimental design and data analysis. All experiments were designed as a split plot with *C. sublineolum* isolates as the main plot and the grain sorghum inbred lines as the split plot. Three plants of each sorghum breeding line were evaluated for each main plot treatment (isolate). Each plant represented a replication and all experiments were repeated once. The main plots consisted of 98 *C. sublineolum* isolates (Table 1) while the split plots consisted of eight grain sorghum inbred lines:

Table 1. (continued from preceding page)

Isolate	Pathotype ^y	Origin		Year	Hybrid
		Location, AR ^z	County		
AR648	7	Pine Tree	St. Francis	2004	Triumph TR82G
AR659	9	Pine Tree	St. Francis	2004	Asgrow A571
AR661	9	Marianna	Lee	2004	Sorgh. Partners K72-J6
AR664	9	Pine Tree	St. Francis	2004	Dyna Gro 780B
AR666	9	Marianna	Lee	2004	Dyna Gro 751B
AR668	9	Pine Tree	St. Francis	2004	Unknown/Field isolate
AR670	9	Pine Tree	St. Francis	2004	Terral TV9421
AR672	7	Pine Tree	St. Francis	2004	Dyna Gro 751B
AR673	9	Pine Tree	St. Francis	2004	Unknown/Field isolate
AR675	8	Pine Tree	St. Francis	2004	Terral TV9421
AR677	9	Marianna	Lee	2004	Dyna Gro 751B
AR683	9	Marianna	Lee	2004	Asgrow A571
AR689	7	Pine Tree	St. Francis	2004	Golden Acres 444E
AR694	9	Marianna	Lee	2004	Dyna Gro 751B
AR699	?	Pine Tree	St. Francis	2004	Pioneer 84G62
AR727	9	Marianna	Lee	2004	Dyna Gro 751B
AR730	9	Pine Tree	St. Francis	2004	DEKALB DK553-11
AR733	9	Marianna	Lee	2004	DEKALB DK553-11
AR735	9	Marianna	Lee	2004	Pioneer 84G62
AR776	9	Pine Tree	St. Francis	2004	Golden Acres 444E
AR777	9	Pine Tree	St. Francis	2004	Triumph TR82G
AR778	9	W of Kelso	Desha	2004	Unknown/Farmer's field
AR779	9	W of Kelso	Desha	2004	Unknown/Farmer's field
AR780	?	W of Kelso	Desha	2004	Unknown/Farmer's field
AR781	9	Near Backgate	Desha	2004	Unknown/Farmer's field
AR783	9	Near Backgate	Desha	2004	Unknown/Farmer's field
AR786	7	Near Backgate	Desha	2004	Unknown/Farmer's field
AR787	9	Near Backgate	Desha	2004	Unknown/Farmer's field
AR793	9	Near Backgate	Desha	2004	Unknown/Farmer's field
AR797	9	S of Marianna	Lee	2004	Unknown/Farmer's field
AR798	9	S of Marianna	Lee	2004	Unknown/Farmer's field
AR800	?	S of Marianna	Lee	2004	Unknown/Farmer's field
AR801	?	N of Biscoe	Prairie	2005	Unknown/Farmer's field 1
AR803	7	N of Biscoe	Prairie	2005	Unknown/Farmer's field 1
AR805	?	N of Biscoe	Prairie	2005	Unknown/Farmer's field 1
AR807	7	N of Biscoe	Prairie	2005	Unknown/Farmer's field 2
AR808	9	N of Biscoe	Prairie	2005	Unknown/Farmer's field 2
AR809	15	Pine Tree	St. Francis	2005	Asgrow A571
AR813	?	Pine Tree	St. Francis	2005	Triumph TR481
AR815	12	Pine Tree	St. Francis	2005	Golden Acres 444E
AR816	9	Pine Tree	St. Francis	2005	Southern States SS-800
AR817	?	Pine Tree	St. Francis	2005	Terral TV9421
AR820	?	Pine Tree	St. Francis	2005	Terral TV96H81
AR825	?	Marianna	Lee	2005	Unknown/Filler row
AR826	9	Near Backgate	Desha	2005	Unknown/Farmer's field
AR828	9	Near Backgate	Desha	2005	Unknown/Farmer's field
AR830	7	Near Backgate	Desha	2005	Unknown/Farmer's field
AR831	?	Marianna	Lee	2005	Monsanto MTC15525
AR834	8	Marianna	Lee	2005	Southern States SS-650
AR837	19	Marianna	Lee	2005	Terral TV93S72
AR838	16	Marianna	Lee	2005	Pioneer 83G66
AR841	?	Marianna	Lee	2005	Triumph TR82-G

SC326-6, SC414-12E, BTX378, TAM428, TX2536, SC328C, QL3 (India), and BTX398.

Disease severity ratings were analyzed using the JMP statistical software package (SAS Institute, Cary, NC). Analyses of the disease severity ratings were conducted based on the locations where the isolates were collected, and three different split-plot analyses were performed. The main parameters used for these analyses were the year isolates were collected, the county in which isolates were collected, and the source in which isolates were collected. Source was used in these experiments to denote isolates collected from producer fields versus isolates collected from research stations. For each main parameter listed above, grain sorghum inbred lines (host differentials) were used as the split parameter. When interaction means were significant, least significant differences ($P = 0.05$) were calculated using the guidelines provided by Little and Hills (8). In the absence of significant interactions, the main effects were compared using the least squares means t tests ($P = 0.05$).

RESULTS

Pathotype designation. Pathotypes 1 through 8 were previously described using *C. sublineolum* isolates from Texas, Georgia, and Puerto Rico (2). A summary of the reactions of the eight host differential sorghum inbred lines to the *C. sublineolum* isolates collected in Arkansas are presented in Table 2. Of the 98 isolates tested, only 87 isolates are reported here because reactions varied between MR and MS ratings (ability to sporulate on the host) on one or more of the host differentials between experiments for the other 11 isolates and, therefore, were excluded from further analysis. Among the remaining 87 isolates,

we identified 13 pathotypes with 53% of the total samples evaluated being designated as pathotype 9, which has not been previously reported. None of the isolates evaluated in these experiments were identified as pathotypes 1 through 6. However, isolates representing pathotypes 7 and 8, which had been previously reported (2), were found in Arkansas, as well as 11 new pathotypes (nos. 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, and 19).

Five pathotypes were found from isolates collected from grain sorghum production fields in four different counties in Arkansas. More importantly, 10 of the 20 isolates evaluated from production fields were classified as pathotype 9. In contrast, 12 pathotypes were discovered from isolates collected from research stations in Lee and St. Francis Counties. However, the

majority of the isolates (35 of 67) evaluated from research stations were designated as pathotype 9, which is similar to the results from isolates collected from grain sorghum production fields.

Anthracnose severity. There was a significant year of isolate collection–host differential interaction ($P = 0.0001$) and a significant interaction ($P = 0.0226$) for the county of isolate collection–host differential on disease severity (Table 3). No significant interactions ($P = 0.7204$) were observed for the source of isolate collection–host differential on disease severity (Table 3). There was a significant main effect from source (producer fields or research stations) on disease severity. Similarly, host differentials had a significant main effect on disease severity; however, variability between the inbred lines was

Table 3. Analysis of variance components including the degrees of freedom, sum of squares, F ratio, and P values for the effect of *Colletotrichum sublineolum* isolates collected by year, county, and from producer fields versus research stations on disease severity of eight grain sorghum host differentials^y

Source of variation ^z	df	Sum of squares	F ratio	P value
Year				
Year	2	135,968.18	3,792.276	0.0001
Host differential	7	235,457.09	137.032	0.0001
Year $\times$ host differential	14	60,651.85	17.649	0.0001
County				
County	3	2,606.46	129.801	0.0001
Host differential	7	187,056.07	78.889	0.0001
County $\times$ host differential	21	12,204.69	1.716	0.0226
Location				
Source	1	1,938.99	286.813	0.0035
Host differential	7	24,073.22	100.842	0.0001
Source $\times$ host differential	7	1,535.46	0.643	0.7204

^y Isolates used in these experiments were collected in 2003, 2004, and 2005 within the state of Arkansas, from Desha, Lee, Prairie, and St. Francis Counties, two University of Arkansas research stations, and from different producer fields within the state. Disease severity ratings were collected 14 days after inoculation. Host differentials used in these experiments were SC326-6, SC414-12E, BTX378, TAM428, TX2536, SC328C, QL3 (India), and BTX398 (2).

^z Isolates were collected by year (Year) and county (County) and from producer fields and research stations (Location).

Table 2. Pathotype designation for 87 *Colletotrichum sublineolum* isolates collected in Arkansas based on virulence patterns for each isolate on eight grain sorghum host differentials

Pathotype	No. of isolates ^z	Differential line							
		SC326-6	SC414-12E	BTX378	TAM428	TX2536	SC328C	QL3	BTX398
1	0	–	–	–	–	–	–	–	+
2	0	–	–	–	–	–	–	+	+
3	0	–	–	+	–	–	–	–	+
4	0	–	–	–	+	+	+	–	+
5	0	–	–	+	+	+	+	+	+
6	0	–	+	+	+	+	+	+	+
7	13	+	–	+	+	+	+	+	+
8	9	+	+	–	+	+	+	+	+
9	46	+	+	+	+	+	+	+	+
10	6	+	+	–	+	+	–	+	+
11	4	+	+	+	–	+	–	+	+
12	2	+	+	–	–	+	+	+	+
13	1	+	–	–	+	+	+	+	+
14	1	+	+	–	–	+	–	+	+
15	1	+	–	–	+	+	–	+	+
16	1	+	–	–	+	+	+	–	+
17	1	+	+	–	+	+	–	+	–
18	1	+	–	–	+	+	–	–	+
19	1	+	–	–	+	+	–	–	–

^z In all, 98 isolates were evaluated in these experiments. However, only 87 isolates are reported here because 11 isolates produced different reactions on some of the differentials between repetitions and therefore were excluded from further analysis.

expected to have a significant influence on disease severity because some of the differential lines were resistant to some of the isolates. Therefore, the results on the effect of host differentials on disease severity pooled over both sources are not reported here.

Isolates collected in 2003 caused significantly higher levels of disease on the differential inbred line TX2536 than on SC326-6, SC414-12E, and BTX378 (Table 4). There were no significant differences for disease severity among differentials TAM428, TX2536, SC328C, QL3, and BTX398 from isolates collected in 2003. SC328C had significantly more disease than SC326-6, SC414-12E, and BTX378 for isolates collected in 2004 (Table 4). Similarly, no significant differences were detected for disease severity on TAM428, TX2536, SC328C, QL3, and BTX398 for the isolates collected in 2004. The results of testing isolates in the 2005 collection produced results similar to the 2003 and 2004 isolates. SC328C was more susceptible than SC326-6, SC414-12E, and BTX378 but there were no significant differences for TAM428, TX2536, SC328C, QL3, and BTX398 (Table 4).

There were significant interactions between the county from which the isolates were collected and the host differentials and there was a similar trend for the amount of disease on each host differential (Table 5). SC326-6 and SC414-12E were more resistant than TAM428 and SC328C for isolates from Desha and Prairie Counties. Numerically, the lowest level of disease was found on SC414-12E for all counties; the amount of disease on SC414-12E was not statistically different from SC326-6, BTX378, QL3, and BTX398 for isolates collected from Desha and Prairie Counties. There were no differences in disease severity on SC414-12E compared with SC326-6, BTX378, TAM428, QL3, and BTX398 for isolates collected in Lee and St. Francis Counties. When comparing the source (producer fields and research stations), isolates collected from research stations caused significantly more disease (27.8%) than the isolates collected from producer fields (25.6%) when pooled over all host differentials.

DISCUSSION

C. sublineolum isolates were collected in grain sorghum production areas from four counties located in the Mississippi River delta region of Arkansas to evaluate the pathogenic diversity of populations in Arkansas. The research reported here demonstrates that diversity, measured as differences in virulence, exists within the *C. sublineolum* populations in Arkansas. Based on virulence to eight host differential inbred sorghum lines, 11 new pathotypes were discovered in Arkansas in addition to the eight previously reported pathotypes (2,4). Pathotype diversity

seemed to be more significantly associated with the year the isolates were collected than with the county from which the isolates were collected. In 2003, 10 of the 13 pathotypes reported in this work were discovered from the different isolate collection sites. In contrast, only 3 of the 13 pathotypes reported here were found in 2004, with isolates representing pathotype 9 being the dominant group.

Pathotype 9 isolates were the majority of isolates at all of our sampling sites in 2004. The source of initial inoculum was probably a mixture of many pathotypes that had previously been found at most of the sites in 2003. Previously, Li (7) showed that one *C. sublineolum* strain was detected at a higher frequency near the end of the growing season, although three strains were equally inoculated on the sorghum plants earlier in the growing season. Xia et al. (18) reported a similar situation with the rice blast pathogen *Magnaporthe grisea* and suggested that a founder effect and surrounding cultivars may have influenced the composition of populations within a susceptible field. Because our isolates were collected near the end of the growing season (after heading), we may have selected for individual isolates instead of multiple populations that could have been present during the earlier part of the growing season. However, isolates were found for pathotypes 13, 14, 15, 16,

17, 18, and 19, suggesting that smaller populations are still present from year to year but at a lower frequency. Previous research reported that *M. grisea* race recovery varied by cultivar, and several races could be recovered from one cultivar (3).

A larger number of pathotypes was found at research stations than in producer fields. One possible explanation for this outcome would be the differences in selection pressure between these two systems. For example, usually only one sorghum hybrid was planted in each producer's field whereas multiple hybrids were planted at research stations. Cardwell et al. (2) reported that care should be exercised because it is not known whether a complex of isolates persists in a population or whether these isolates are a threat outside of research stations. It has been suggested that sorghum cultivars should be thoroughly screened against the *C. sublineolum* races and pathotypes prevalent to an area before hybrids are released for large-scale production (1).

The virulence of each isolate was determined by comparing susceptible reactions across different host differentials, but the level of disease varied between susceptible reactions. Nine sorghum isolates of *C. sublineolum* collected from different locations in India were grouped into three distinct aggressiveness categories based on the reactions of six susceptible genotypes

Table 4. Comparison of *Colletotrichum sublineolum* isolates collected in different years on the severity of sorghum anthracnose for eight sorghum host differentials

Host differential	Disease severity (%) ^z		
	2003	2004	2005
SC326-6	9.9 b	16.5 cd	11.2 abc
SC414-12E	8.5 b	6.3 d	4.6 c
BTX378	8.9 b	25.0 bcd	9.5 bc
TAM428	22.8 ab	47.9 ab	31.4 ab
TX2536	37.4 a	47.5 ab	24.7 abc
SC328C	20.6 ab	58.9 a	35.0 a
QL3 (India)	24.1 ab	41.6 abc	15.9 abc
BTX398	23.3 ab	42.6 ab	21.2 abc

^z Values are the least squares means of all isolates collected in the designated year. Disease severity ratings were collected 14 days after inoculation, and means within the same year followed by the same letter are not significantly different ($P = 0.05$). Least significant difference (0.05) to compare host differentials across years = 23.9.

Table 5. Effect of *Colletotrichum sublineolum* isolates collected in different counties in Arkansas on the severity of sorghum anthracnose for eight sorghum host differentials

Host differential	Disease severity (%) ^z			
	Desha	Lee	Prairie	St. Francis
SC326-6	11.8 bc	13.5 ab	13.6 bc	13.6 bc
SC414-12E	4.1 c	7.0 b	5.2 c	6.9 c
BTX378	18.5 abc	13.4 ab	16.0 bc	20.6 bc
TAM428	44.6 a	33.6 ab	48.7 a	36.0 abc
TX2536	36.7 ab	37.7 a	37.1 ab	42.4 ab
SC328C	42.3 a	40.0 a	49.8 a	43.9 a
QL3 (India)	30.0 abc	30.0 ab	21.9 abc	32.8 abc
BTX398	25.6 abc	33.4 ab	34.5 abc	32.0 abc

^z Values are the least squares means of all isolates collected in the designated counties. Disease severity ratings were collected 14 days after inoculation, and means within the same county followed by the same letter are not significantly different ($P = 0.05$). Least significant difference (0.05) to compare host differentials across counties = 27.9.

(10). In our experiments, we found two significant interactions. The first was for the year the isolates were collected and the host differential. The second was for the county from which isolates were collected by host differential on disease severity for the eight sorghum differential inbred lines previously used to describe pathotypes (2,4). Isolates collected from the research stations tended to cause more disease than the isolates collected from producer fields when the data were pooled over all host differentials. All Arkansas isolates evaluated in these experiments were virulent to SC326-6 and TX2536 and more than 50% of the isolates were virulent to all of the host differentials tested, suggesting that these inbred lines would not be good parents for breeding for anthracnose resistance in Arkansas.

Overall, our isolates caused the least amount of disease on SC326-6 and SC414-12E. It was also noted that lesions formed only on the midvein of SC414-12E and not on the leaf lamina. This was not as evident for SC326-6; however, more lesions seemed to be on the midvein for this inbred line as well, although lesion numbers were not collected for these experiments. This type of infection could have a tremendous effect on the percentage of diseased area. The remaining six inbred lines had lesions on both the leaf lamina and midvein, producing more diseased area.

To our knowledge, the 11 new pathotypes identified in this study have not been previously reported using these host differentials. Isolates in pathotype 9 are virulent to all eight host differentials and comprised 53% of the total samples evaluated. More experiments are needed using different sorghum inbred lines to determine whether resistance exists for these isolates. If more inbred lines are incorporated into

future pathotype experiments, then more pathotypes may be discovered within *C. sublineolum* populations found in Arkansas. In addition, more experiments are needed to evaluate the effect of pathotype 9 on commercial grain sorghum hybrids. This information should be useful to sorghum breeders and producers in order to effectively manage sorghum anthracnose in Arkansas.

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