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PATHOGENICITY, AGGRESSIVENESS, AND VIRULENCE OF THREE SPECIES OF *CERCOSPORA* ASSOCIATED WITH GRAY LEAF SPOT OF MAIZE [1]

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ABSTRACT - In the eastern United States, three genetically distinct species of *Cercospora,* the two sibling species of *C. zeae-maydis* (types I and II) and *C. sorghi* var. *maydis*, are routinely isolated from maize leaves exhibiting symptoms of gray leaf spot (GLS). To determine if resistance to GLS is species specific, we conducted field trials comparing four commercially available maize hybrids for resistance to four isolates of *C. zeae-maydis* type I, three isolates of *C. zeae-maydis* type II, two isolates of *C. sorghi* var. *maydis*, as well as isolates of *C. sorghi* from *Sorghum* spp., and *C. kikuchii* from soybean. Isolates within each of the two sibling species of *C. zeae-maydis* were highly variable in their aggressiveness on the hybrids. Resistance in maize to GLS appears to be equally effective against both type I and type II of *C. zeae-maydis*. Neither of the two isolates of *C. sorghi* var. *maydis* isolated from maize, the *C. sorghi* isolates, nor *C. kikuchii* were pathogenic on maize. These results indicate that aggressive isolates, regardless of which sibling species of *C. zeae-maydis*, should be used to select for GLS resistance in field trials.

KEY WORDS: Resistance; Gray leaf spot; *Cercospora*.

INTRODUCTION

Gray leaf spot (GLS) is considered to be the most important foliar disease of maize in the United States. The disease was originally described from southern Illinois in 1925 (Tehon and Daniels, 1925), but for many years it was considered to be a relatively obscure and unimportant disease (Roane, 1950; Laterell and Rossi, 1983). Since the 1970's, the disease has spread and increased in incidence and severity as reduced tillage practices have become commonplace in the eastern and Midwestern states (Laterell and Rossi, 1983). GLS is now widely distributed from eastern Colorado (under center pivot irrigation) to coastal regions of North Carolina and Virginia (Dunkle and Carson, 1999).

Gray leaf spot is caused by the imperfect fungus, *Cercospora zeae-maydis* Tehon & Daniels, although *C. sorghi* var. *maydis* is also listed as being associated with GLS lesions (Chupp, 1953). The pathogen, *C. zeae-maydis* is composed of two genetically distinct, but morphologically similar sibling species (Carson *et al*.., 1997; Wang *et al*., 1998). The more common sibling species (Type I) is widely distributed in the U.S., China, and Latin America, while the second sibling species (Type II) is confined to the eastern U.S. and Africa (Dunkle and Levi, 2000). Variability in pathogenic aggressiveness among isolates of *C. zeae-maydis* was a cause of significant genotype X environment interactions in GLS trials in North Carolina (Carson *et al*., 2002). In that study, only a single, non-aggressive isolate of *C. zeae-maydis* type II, was compared to several isolates of *C. zeae-maydis* type I of variable aggressiveness. A previous study (Bair and Ayers, 1986) revealed considerable variation in aggressiveness among isolates of *C. zeae-maydis*, but it is not known whether both two sibling species were used in that work. *C. sorghi* var. *maydis* is also associated with GLS lesions in the southern U.S., but its pathogenicity on maize is not clear (Chupp, 1953). Recent molecular studies of the genus *Cercospora* showed *C. sorghi* var. *maydis* to be phylogenetically distinct from *C. sorghi* and *C. zeae-maydis* (Goodwin *et al*., 2001).

Genetic resistance is the primary means of control of GLS in the U.S. Sources of resistance have been found among temperate and tropical germplasm, and this resistance has been incorporat-

[1] This paper is dedicated to a good friend and colleague, Major Goodman, in recognition of his many contributions to maize genetics, germplasm preservation and utilization, and maize improvement.

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ed into maize breeding programs (THOMPSON *et al.*, 1987; ULRICH *et al.*, 1990; DONAHUE *et al.*, 1991; BUBECK *et al.*, 1993; CLEMENTS *et al.*, 2000). Commercial hybrids with acceptable levels of GLS resistance are now available in many areas of the U.S. Most GLS resistance in commercial hybrids is coming from the male inbred line parent (or 'Lancaster') side of the hybrid pedigree. The objectives of this study were to determine the pathogenicity, aggressiveness, and virulence of representative isolates of the two sibling species of *C. zeae-maydis* and *C. sorghi* var. *maydis* on different sources of resistance.

MATERIALS AND METHODS

Four maize hybrids, Dekalb DK683, LH132 x NC258, FFR406.411xLH214, and Pioneer hybrid 3394, were evaluated against 11 isolates of *Cercospora* in field trials at the Central Crops Research Station on the upper coastal plain of North Carolina at Clayton over the 1998 and 1999 growing seasons. GLS has not been observed on this research station, and the fields used were 'clean tilled" and had not been cropped in maize for the two previous growing seasons. DK683, LH132 x NC258, and FFR406.411 x LH214 were selected because they represent different sources of resistance to gray leaf spot and are commercial hybrids. Pioneer hybrid 3394 was selected as a susceptible check and because it was one of the most widely grown hybrids in the U.S. in the mid 1990's.

The isolates evaluated in the field trial are listed in Table 1. In 1998, only a single isolate of *C. sorghi* var. *maydis* (NC-5) was evaluated. In 1999, a second isolate of *C. sorghi* var. *maydis*, NC-6, was substituted for the single isolate of *C. kikuchii* (NC-8).

The field trial consisted of a factorial design with three replications arranged in a randomized complete block design with isolates and hybrids constituting the main factors. Plots were separated from each other by at least 2 m of uninoculated border plots of the highly resistant hybrid DK683. Plots were inoculated with a sorghum grain culture of the *Cercospora* isolates twice; at the four-six and at the eight-ten leaf stages of development. The sorghum grain inoculum was prepared by soaking sorghum seed overnight in tap water, draining off excess water, dispensing the soaked grain into 1 L flasks, and autoclaving for 1 hr. Flasks were inoculated with 20 ml of a slurry made by agitating 10 to 14 day old V-8 agar cultures of the isolates in sterile water with 5 mm glass beads. The concentration of conidia and hyphal fragments in the slurry was not determined. The sorghum grain cultures were grown at room temperature until the sorghum seed was thoroughly colonized (~ 3 wks), and were stored at 4∞ until used. Whole plots were visually rated for percent GLS severity five times, beginning at mid-anthesis in 1998; three times beginning two weeks post-anthesis in 1999. Areas under the disease progress curve (WILCOXSON *et al.*, 1974) were calculated and subjected to analysis of variance. Because isolates of *C. sorghi* var. *maydis, C. sorghi,* and *C. kikuchii* were not pathogenic on maize in these trials, they were not included in the analysis. The variation among isolates of the two sibling species of *C. zeae-maydis* was partitioned into variation within and between them. Similarly, the hybrid x isolate term in the ANOVA was partitioned into hybrid x isolate interaction within each of the species and the hybrid x species interaction.

RESULTS AND DISCUSSION

Gray leaf spot development was excellent in both years of the study, reaching a high of over 75 and 85% severity by the last rating date in some plots of Pioneer 3394 in 1998 and 1999, respectively. There was substantial variation in aggressiveness among isolates within each of the two sibling species of *C. zeae-maydis* (Table 2) in each year of the study. The isolate x year interaction was highly significant, mainly due to the loss of aggressiveness of the IL-1 isolate in 1999 compared to 1998. Although the exact cause of this apparent loss of aggressiveness is unknown, it is not uncommon for isolates of *C. zeae-maydis* of to lose aggressiveness and the ability to sporulate when maintained by repeated subculturing (LATERELL and ROSSI, 1983). The relative ranking of the remaining isolates was the same over both years of the trial. Hybrid x isolate interactions within each of the sibling species in the combined analysis was significant. As reported previously (CARSON *et al.*, 2002), the interaction arose from the inability of less aggressive isolates to dis-

TABLE 1 - *Isolates of* Cercospora *species evaluated in field trials at Clayton, NC.*

Species	Isolate	Source
C. zeae-maydis Type I	IL-1	Edgar Co., IL
C. zeae-maydis Type I	NC-1	Hendersonville, NC
C. zeae-maydis Type I	IN-1	Indiana[1]
C. zeae-maydis Type I	NC-2	Marion, NC
C. zeae-maydis Type II	NC-3	Fletcher, NC
C. zeae-maydis Type II	NC-4	Laurel Springs, NC
C. zeae-maydis Type II	PA-1	Oley, PA[1]
C. sorghi var. *maydis*	NC-5	Davidson Co., NC
C. sorghi var. *maydis*	NC-6	Stanly Co., NC
C. sorghi	NC-7	Rowan Co., NC[2]
C. sorghi	TX-1	Texas[1]
C. kikuchii	NC-8	North Carolina[3]

[1] Culture kindly provided by L. Dunkle, USDA-ARS, West Lafayette, IN.
[2] Isolated from Johnsongrass (*Sorghum halepense*) in a maize field.
[3] Culture kindly provided by R.G. Upchurch, USDA-ARS, Raleigh, NC.

TABLE 2 - *Mean area under the disease progress curve (AUDPC) for gray leaf spot on four hybrids caused by* Cercospora *spp. isolated from maize as well isolates of* C. sorghi *and* C. kikuchii.

Isolate	Hybrid							
	LH132xNC258		DK683		(FFR406x411)xLH214		Pioneer3394	
	1998	1999	1998	1999	1998	1999	1998	1999
C. zeae-maydis Type I								
IL-1	139	34	92	38	436	82	520	214
NC-1	16	44	23	71	36	118	72	176
IN-1	6	18	18	12	20	62	34	106
NC-2s	229	637	164	432	447	775	675	1138
C. zeae-maydis Type II								
NC-3	95	147	77	129	210	442	335	850
NC-4	12	7	15	10	30	12	77	74
PA-1	138	407	97	320	242	606	404	1247
C. sorghi var. *maydis*								
NC-5	not pathogenic							
NC-6	not pathogenic							
C. sorghi								
NC-7	not pathogenic							
TX-1	not pathogenic							
C. kikuchii								
NC-8	not pathogenic							

TABLE 3 - *Mean squares for area under the disease progress curve for four maize hybrids inoculated with isolates of two sibling species of* Cercospora zea-maydis (*CzmI & CzmII*) *in field trials at Clayton, NC.*

Source of variation	df	1998	1999	combined
Hybrids	3	245199**[a]	659909**	848786**
Isolates	6	241942**	1088058**	1033361**
Isolates/CzmI	3	396568**	1319908**	1378042**
Isolates/CzmII	2	115631**	1163853**	998804**
CzmI vs CzmII	1	30687	21233	42738
Hybrids x Isolates	18	25952**	82497**	79300*
Hybrid x Species	3	4832	105510**	45458
Hybrid x Isolate/CzmI	9	43513**	44579**	74210*
Hybrid x Isolate/CzmII	6	10169	127910**	104351*

[a] *, ** indicate mean square is significant at the 0.05 and 0.01 level of probability, respectively.

criminate subtle levels of resistance among the four hybrids and not due to any dramatic changes in the ranking of the resistance of the hybrids by the different isolates. Variation in aggressiveness among isolates within each of the sibling species was much more important than any differences between them (Table 3). There was no significant hybrid x species interaction in the combined analysis, indicating that hybrids resistant to one species are resistant to the other. Symptoms produced by the two sibling species on maize appeared to be typical of gray leaf spot and we could not discriminate between them.

Neither of the two isolates of *C. sorghi* var. *maydis* isolated from maize were pathogenic in our trials, nor were isolates of *C. sorghi* from johnsongrass or sorghum and *C. kikuchii*. We routinely reisolated *C. sorghi* var. *maydis* from leaf tissue sampled from inoculated plots, but the fungus appeared to arise as a saprophyte. It was associated with GLS lesions caused by either of the two sibling species of *C. zeae-maydis,* southern leaf blight lesions, pustules of common rust, or areas of necrotic tissue arising from physical injuries to the leaf. Because *C. sorghi* var. *maydis* grows rapidly, it is often the first *Cercospora* species to sporulate on maize leaf tissue placed in moist chambers if it is present. Our results do not preclude the possibility that isolates of *C. sorghi* var. *maydis* pathogenic on maize may be found with more extensive sampling of isolates, but our observations suggest it is an efficient saprophyte on maize leaf tissue.

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