Gray Leaf Spot of Corn: Yield Loss and Evaluation of Germplasm for Resistance

by

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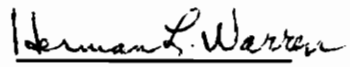

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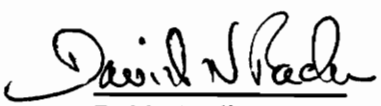

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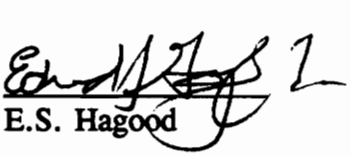

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ABSTRACT

Gray leaf spot (GLS) of corn (*Zea mays* L.), caused by the fungus *Cercospora zeae-maydis* (CZM) (Tehon and Daniels) has increased in incidence and severity with increasing use of no-tillage and continous corn practices. This disease can be yield limiting. Corn hybrids were evaluated under natural disease pressure for three years (1989, 90, and 91) at two locations (Montgomery and Wythe Co., VA). Yield losses ranged from 2127.4 kg/ha (Wythe Co., 1991) to 4242.2 kg/ha (Wythe Co., 1990). It was estimated that 77% of the variability in yield was due to GLS. Fungicides were evaluated for the control of GLS over three years on a susceptible hybrid, Pioneer Brand 3320. All fungicides, with the exception of mancozeb, provided significant control over nontreated check in all years. Benomyl, propiconazole and terbutrazole were the most effective fungicides. As much as 93% of the variablilty in yield was attributed to blighting. Reduction in blighting also increased the kernel weight. The toxin, cercosporin, produced by CZM was evaluated for its ability to elicit differential responses

in corn germplasm by three methods, *ie.*, vein inoculation, root, and shoot uptake. No consistant differential reponses were found with vein inoculation, but 31-day old plants were significantly more sensitive to the toxin than 21-day old plants, as measured by lesion width. Root and shoot uptake of the toxin by inbred germplasm produced lesions that resembled those produced by CZM in the field. Microscopic, yellow fluorescing crystals were found associated with necrotic tissue from toxin-treated inbreds. Significantly more injury occurred to toxin-treated inbreds exposed to light than to darkness. By chromatographic analysis, 407.1-1076.7 ng of toxin/g of tissue was recovered from leaf lesion extracts of plants exposed to light. Five inbreds (B73, H99, Va59, NC250a, and NC264) showed consistent and differential responses to the toxin. H99 and NC250a showed differential responses to the same concentration of toxin, thus suggesting that some germplasm are more sensitive to the toxin than others. Tests using the toxin as a means to identify resistant germplasm did not provide reliable predictions of germplasm response to CZM in the field.

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INTRODUCTION AND OBJECTIVES

Gray leaf spot (GLS) of corn (*Zea mays* L.), a disease caused by the fungus, *Cercospora zeae-maydis* (Tehon and Daniels, 1925), has become a significant threat to corn production in many areas in Virginia and sporadically in major corn producing regions of the midwest. It's increasing frequency and severity has been associated with methods of corn production that use reduced or no-tillage (Beckman *et al.*, 1981; Beckman and Payne, 1982; Hilty *et al.*, 1979; Kingsland, 1963; Roane *et al.*, 1974; Roane, 1950; Rupe *et al.*, 1979; Stromberg, 1984). The disease may cause between 10 and 25% yield loss annually in problem areas. Experimental studies with susceptible hybrids have shown losses of 50% and higher (Stromberg, 1990a, 1990b; Stromberg and Carter, 1991a, 1991b; Stromberg and Donahue, 1986).

Losses caused by GLS are due to premature loss of photosynthetic tissue. Blighting begins on the lower leaves of the corn plant when weather conditions are favorable; *eg.*, periods of high relative humidity and leaf moisture. Blighting progresses up the corn plant and, if severe, causes a significant reduction in photosynthate required for grain fill. Depending on severity, growth stage, and hybrid, losses in grain yield may range from 313.8 to 3137.8 kg/ha. (Stromberg, 1990a, 1990b; Stromberg and Carter, 1991a, 1991b; Stromberg and Donahue, 1986).

Preliminary studies of gray leaf spot by Ayers *et al.* (1984) and Manh (1977) indicated that resistance was dominant and quantitatively inherited. Huff *et al.* (1988) found that GLS resistance was quantitatively inherited, highly heritable and dominant. Donahue (1989) developed a complete diallel cross of fourteen corn inbreds to determine

the inheritance of gray leaf spot disease and confirmed that it is highly heritable, not complex, and additive. This supported studies done previously by Thompson *et al.* (1987).

The overall goal of this research is to develop methods to evaluate germplasm for resistance or susceptibility to *C. zeae-maydis*. Specific objectives are: 1) to evaluate the level of GLS resistance in commercially available hybrids, 2) to evaluate the efficacy of foliar fungicides to control GLS on corn, assess the degree of blighting, and correlate blight severity with yield reduction, and 3) to determine the value of the reaction of seedling germplasm to purified cercosporin toxin, produced by *C. zeae-maydis,* in predicting field resistance to GLS disease.

LITERATURE REVIEW

Distribution and Disease Severity

Gray leaf spot of corn occurs in the mid-Atlantic region of the United States along river bottom lands in the mountain and Piedmont areas from Pennsylvania to South Carolina (Thompson *et al.*, 1987). This disease occurs in many areas of the midwest, and also has been found in western Kansas and Nebraska (Smith, personal communication). Distribution of *C. zeae-maydis* and severity of gray leaf spot have increased since 1970 with the increase in use of minimum tillage farming practices (Hilty *et al.*, 1979 and Roane *et al.*, 1974).

The most severe gray leaf spot disease development is observed in areas where

slow-drying dew and lingering late season fogs result in extended periods of high relative humidity and leaf wetness (Beckman and Payne, 1982, 1983); but the disease is not restricted to these areas when abundant initial inoculum is present (Latterell and Rossi, 1983). Rupe (1982) determined the influence of environment on disease development in Kentucky. Locations where gray leaf spot occurred had many days with high relative humidity and leaf wetness. These conditions appear to be required for high numbers of conidia to be released and for infection to occur.

Disease Cycle/Life cycle of Pathogen

Initially, gray leaf spot leaf lesions are small in size, irregular to rectangular in shape and brown surrounded by a yellow halo. These lesions are first bounded by larger veins, but later coalesce and reduce photosynthetic area until part or all of the leaf becomes necrotic. Under natural conditions of colonization, lesions are first observed on the lower leaves. As these lesions develop and produce conidia they provide inoculum for infection of upper leaves.

Conidia of *C. zeae-maydis* are slightly curved, hyaline, and multiseptate. They are supported on conidiophores emerging from leaf blades, sheaths, and husks. *C. zeae-maydis* is a poor soil competitor, and conidia overwinters on infested corn debris. (Stromberg and Donahue, 1986; Latterell and Rossi, 1983).

Cercosporin

Cercosporin [1,12-bis(2-hydrozypropyl)-2,11-dimethoxy-6,7-methylene-dioxy-4,9-dihydroxyperylene-3,10-quinone] is a light activated, free-radical producing

toxin produced by members of the genus *Cercospora*. It's structure was determined independently by Lousberg *et al.* (1971) and Yamazaki and Ogawa (1972) and was first isolated in 1957 (Kuyama) from *Cercospora kikuchii*, a soybean pathogen. Cercosporin causes necrotic lesions on a wide range of host species (Daub, 1982). When exposed to light, the toxin forms excited states that transfer light energy to oxygen, producing both singlet oxygen and superoxide (Daub and Hargarter, 1983). These compounds are toxic to living cells by oxidizing lipids, proteins, carbohydrates and nucleic acids (Daub, 1987; Spikes, 1977). Plant cell damage is caused by membrane lipid peroxidation and changes in membrane structure when cells are exposed to cercosporin in the presence of light (Fore, 1988). Light is required for development of symptoms and damage to plant tissue (Calpouzos, 1966; Calpouzos and Corke, 1963; Calpouzos and Stallknecht, 1967). Macri and Vianello (1979) reported that cercosporin required thirty minutes exposure to an incandescent bulb to induce ion leakage from potato, carrot, beet, and corn tissues. Tobacco leaf discs treated with cercosporin showed a rapid increase of electrolyte leakage after irradiation with a 750 w tungsten projector lamp (Daub, 1982). Steinkamp *et al.* (1981), working with sugar beets concluded that cercosporin played a role in the spread of the fungus through plant tissue. The fungus does not colonize live tissue, but requires the toxin to kill tissue before colonization can take place (Steinkamp et al., 1981). This suggests a critical involvement of cercosporin in disease development. Two facts link cercosporin to the diseases caused by *Cercospora* spp. One is that light is required for symptom development in *Cercospora* incited diseases (Calpouzos, 1966; and Corke,

1963; Calpouzos and Stallknecht, 1967). Secondly, cercosporin produces changes similar to those caused by *C. beticola* in sugarbeet (Steinkamp et al., 1979, 1981).

Not only may cercosporin play an important role in pathogenesis, but it may also play an important role in identifying resistance mechanisms in host species. Paraquat, a light activated, free-radical producing herbicide, has a mode of action similar to cercosporin. Superoxide dismutase (SOD), an enzyme in the photoscavenging cycle, has been shown to reduce stress due to free-radical production and oxidative stress (Rabinowitch and Fridovich, 1983; Matters and Scandalios, 1986). Shaaltiel and Gressel (1986) reported that elevated levels of three enzymes coordinately detoxify free radicals in response to paraquat. These enzymes are superoxide dismutase, ascorbate peroxidase and glutathione reductase. In some weeds resistant to paraquat, these enzymes were found at elevated levels. Since paraquat and cercosporin have similar modes of action, this may suggest a role for SOD's as a mechanism for resistance to GLS.

Gwinn *et al.* (1987) investigated the relationship of cercosporin to gray leaf spot development. Three cultivars of corn varying in resistance were grown in the greenhouse. Leaf disks from 1-, 2-, and 3-month old plants were treated with 1.2 μM cercosporin. Cercosporin treatment caused significantly less ion leakage from disks of older plants and no varietal differences were detectable for sensitivity to cercosporin. Gwinn *et al.* used three genotypes in their study, and one was an inbred line. Since, a wide range of genotypes was not represented, a negative correlation between genotypes and cercosporin sensitivity may not have been warranted. Also, corn plants used in this

experiment were grown in 15-cm pots in the greenhouse during the winter without providing supplemental light. Plants grown under such conditions may not be indicative of those grown in the field during the growing season and may not react to cercosporin similarly to that of field grown plants.

Yield Losses

Ayers *et al.* (1984) documented yield losses from gray leaf spot and studied the inheritance of resistance in adapted germplasm. Various fungicides were used to establish losses due to gray leaf spot. Corn plants treated with mancozeb applied every seven days gave the highest yield. This response to mancozeb treatment may have resulted not just from fungal control but from foliar feeding of zinc and manganese in the fungicide, possibly accounting for part of its effect on yield. The use of mancozeb increased yield by 20% over the nontreated check.

Both inbreds and hybrids have been evaluated for gray leaf spot resistance in the field. Studies done by Donahue (1989) and Donahue *et al.* (1991) on specific combining ability in diallel crosses to corn inbreds indicated that resistant genotypes could easily be selected in a segregating population, thus corroborating with work of Thompson *et al.*, (1987); Ayers *et al.*, (1984); Huff *et al.*, (1988); Manh, 1977. Inbred lines of Va14, Va17, Va85 and KB1250 were newly identified as potential sources of resistance for breeding programs. Donahue's (1989) crosses between resistant inbreds indicated that the nature of resistance was additive.

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Hybrid Performance and Yield Associated with Gray Leaf Spot of Corn

by

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ABSTRACT

Elite corn hybrids were evaluated under heavy natural gray leaf spot (GLS) disease pressure for three years at two locations (Montgomery and Wythe Counties, Virginia). Plants were scored for disease on a Disease Severity Index (DSI) (0-5) and percent lodging, grain moisture and yield were determined. Performance of tolerant hybrids was estimated to be $\geq$ 1.2 times greater than the susceptible hybrid check (Pioneer Brand 3320). The hybrid check consistently had high DSI's and low yield for all locations and years. Hybrid yields ranged from 2698.5-9018.0 kg/ha with significant differences among hybrids. For hybrids in common over years and locations, DSI and yield differed significantly ($P \leq 0.0001$) by location, year, hybrid and rating interval. A comparison of DSI at mid-season and yield at both locations over all years combined, indicated that 77% of the variation in yield was attributed to GLS. Regression analysis of the mid-season DSI and yield at separate locations showed that lower yields at the Montgomery County site ($R^2 = 0.84$) were due to a greater DSI than at the Wythe County site ($R^2 = 0.75$). A tolerant hybrid, Pioneer Brand 3136, consistently yielded higher and had lower DSI's than the susceptible hybrid, Pioneer Brand 3320, even though rainfall patterns differed by year and location. This study demonstrates that environmental conditions interact with GLS development and yield ($P \leq 0.0001$), but evaluation of hybrids under heavy GLS pressure is useful to predict effect on yield and demonstrates that generally more resistant hybrids have higher yields, regardless of location or year.

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Gray leaf spot (GLS) of corn (*Zea mays* L.), a disease caused by the fungus, *Cercospora zeae-maydis* (Tehon and Daniels, 1925), has increased in incidence and severity throughout the mid-Atlantic and south-eastern states and sporadically in regions of the Corn Belt of the midwest. Since 1970, GLS has been observed earlier in the growing season with greater severity (Hilty et al., 1979; Leonard, 1974; Roane, et al., 1974). This increase in disease is associated with the use of minimum tillage and continuous corn practices (Hilty *et al.*, 1979 and Roane *et al.*, 1974; Stromberg, 1984, 1985).

GLS severity has also been associated with periods of high relative humidity, overcast skies, and prolonged leaf wetness (Rupe, *et al.*, 1982; Beckman and Payne, 1982, 1983), however, GLS is not restricted to these areas when abundant inoculum is present (Latterall and Rossi, 1983). Beckman and Payne's (1982,1983) greenhouse studies showed that the pathogen requires high moisture to penetrate and colonize corn leaves. Lesion development occurred only after inoculated plants were kept under these conditions for two weeks or longer depending on plant or tissue maturity. Under field conditions, it is postulated that relative humidity and prolonged leaf wetness are probably the most critical factor in promoting disease severity (Latterall and Rossi, 1983). However, the pattern of climatic conditions associated with severe damage to gray leaf spot disease have not been fully explained.

Losses to gray leaf spot disease are due to premature damage of tissue and subsequent loss of photosynthetic area. Blighting progresses acropetally and when severe,

causes a significant reduction in photosynthate required for grain fill. The disease may cause between 10 and 25% yield loss (Stromberg and Donahue, 1986; Ayers, *et al.*, 1984; and Hilty, *et al.*, 1979). Experimental studies with susceptible hybrids have shown losses of 50% and higher (Stromberg, 1990a, and 1990b; Stromberg and Carter, 1991a, and 1991b).

Host plant resistance is one of the most effective and economical means of preventing yield losses in corn due to GLS. Resistant genotypes have been reported (Ayers, *et al.*, 1984; Bowman, 1983; and Hilty, *et al.*, 1979.), however, none are immune or highly resistant. Currently available hybrids display a range of resistance and susceptiblity to GLS dependent upon the presense of factors required for disease development. Hybrids considered to be resistant may develop significant levels of disease when under conditions favorable to disease development (Ayers *et al.*, 1984; Hilty *et al.*, 1979; Latterell and Rossi, 1983; Roane *et al.*, 1974; Stromberg and Donahue, 1986).

The purpose of this study was to evaluate commercial corn hybrids for their level of blighting and yield under natural infection from *C. zeae-maydis* over three years at two locations conducive to disease development; (*ie.*, Montgomery and Wythe County, Virginia), in order to determine their relative resistance or susceptibility to GLS.

MATERIALS AND METHODS

A total of 47 commercial hybrids obtained from several seed companies were evaluated at two locations over three growing seasons. The first location, in Montgomery County, VA at the University's Whitethorne-Kentland Plantation Experimental Farm, had

been initally infested with *C. zeae-maydis* in 1987. This site, with a deep alluvial soil profile, is located on a terrace adjacent to the New River and consistantly has long periods of high humidity and fogs during night and morning hours that favor gray leaf spot disease development. The second location in Wythe Co., VA on a farmer cooperator's field, has had heavy GLS disease pressure that has been maintained by continuous no-tillage practices since 1965.

The experimental design was a randomized complete block, replicated four times with plots consisting of (7.62 m) rows spaced 0.76 m apart. Seeds were planted at a rate of 52800 seeds/ha. Prior to planting, atrazine (1.48 kg ai/ha) and metolachlor (1.86 kg ai/ha) were applied to the soil surface for weed control. Nicosulfuron (0.035 kg ai/ha), plus 0.25% (v/v) non-ionic surfactant was applied postemergence at approximately the 10th leaf stage for control of johnsongrass at the Montgomery County location. A complete commercial fertilizer (47-23-30 NPK) was applied at 369.9 kg/ha, with the exception of the Wythe County site in 1989 (493.2 kg/ha) as directed by soil testing.

The two middle rows from each plot were scored three times during the growing season for blighting on a Disease Severity Index (DSI) of zero to five as described by Roane *et al.* (1974) and expanded by Hilty *et al.* (1979). For this index, 0 = no symptoms, 1 = trace of lesions below the ear but no lesions above the ear, 2 = many lesions below the ear but only a trace of lesions above the ear, 3 = severe lesion development below the ear and some lesions on leaves above the ear, 4 = all leaves show severe lesion development but green tissue is still visible, and 5 = all leaves are dead.

Percentage lodging, grain moisture, and grain yield expressed at a standard 15.5% moisture were determined for each hybrid in each growing season and significant differences were identified with Duncans Multiple Range Test. Analysis of variance (ANOVA) was completed for each growing season and location separately. Six hybrids common to both locations for all three years were combined for statistical analysis. Dependent variables, *ie.*, grain yield, lodging and grain moisture were analyzed with ANOVA using this general linear model (GLM):

$$\text{Dependent variable} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + e_{ijkl};$$

where i = 1,2 location, j = 1,2,3 year, k = 0,1,2,3,4,5 hybrid (0 = susceptible hybrid, Pioneer Brand 3320), and l = 1,2,3,4 replications. The dependent variable, disease severity, was similarly analyzed by the following GLM:

$$\text{Dependent variable} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \delta_{ijkl} + e_{ijklm};$$

where i = 1,2 locations, j = 1,2,3 years, k = 0,1,2,3,4,5 hybrids, l = 1,2,3 rating dates, m = 1,2,3,4 replications. The effect of GLS on lodging, grain moisture, and grain yield was estimated by the linear regression model:

$$Y = B_o + B_i X_1 + E_i,$$

where Y is the response variable, B_o is the intercept, B_i is the slope of the regression line, X_1 is the regressor variable, and E_i is the extraneous variable.

RESULTS AND DISCUSSION

Environmental conditions affecting the development of GLS disease were variable

depending on location and year. Conditions affecting both locations ranged from a rather wet season in 1989 to an unseasonably dry and hot period from July to September in 1991. In 1990, rainfall early in the season was sufficient for hybrid growth but high temperatures and sparse to moderate rainfall late in the season seemed to retard GLS development. Combined analysis of variance from both gray leaf spot ratings and yields resulted in a significant ($P \leq 0.0001$) interaction between location and year (Tables 1 and 2). For these reasons, each year and site combination was analyzed separately.

Table 1. Combined analysis of variance of gray leaf spot rating from hybrid evaluations grown in Montgomery and Wythe Counties, Virginia, in 1989, 90, and 91.

Source of variation	df	F value	P value
Location	1	42.19	0.0001
Year	2	254.78	0.0001
Hybrid	5	22.93	0.0001
Rating Date	3	2680.48	0.0001
Location * Year	2	263.3	0.0001
Error (means square=0.058)	327		
R^2=0.97			
CV=7.02			

Table 2. Combined analysis of variance of grain yield data (kg/ha) from hybrid evaluations grown in Montgomery and Wythe Counties, Virginia, in 1989, 90, and 91.

Source of variation	df	F value	P value
Location	1	20.04	0.0001
Year	2	37.09	0.0001
Hybrid	5	17.03	0.0001
Location * Year	2	86.70	0.0001
Hybrid * Year	10	3.03	0.0026
R^2=0.85			
CV=14.14			

GLS lesion development was apparent on lower leaves as early as June and progressed up to the ear leaf by late-July. Differences in blighting among hybrids were usually detected by early milk stage (mid-late August) over the three years. Variations include earlier symptom expression of GLS in 1989 and later detection of blight in Wythe County, 1991 (Table 3 & 8).

The best yields were obtained with low levels of blighting, *ie.*, Hytest HT 650A (7580.9 kg/ha), Pioneer Brand 3140 (8183.3 kg/ha), and Pioneer Brand 3154 (6407.3 kg/ha) at Montgomery County, Virginia in 1989, 1990, 1991, respectively. Similar results can be seen with hybrids used in Wythe County, Virginia. Yield of tolerant hybrids was estimated to be at least 1.2 times greater than the check (Pioneer Brand 3320) (Tables 3-8).

Variance between blighting, as quantified by DSI, and yield at Montgomery and Wythe Counties was evident. Yields in 1989 between locations were affected by hurricane Hugo, resulting in more lodging in Wythe than in Montgomery County (Table 3 and 4), that interfered with mechanical harvesting of the test. In 1990, Pioneer Brand 3140 appeared more tolerant to GLS than Pioneer Brand 3320 in both locations (Table 5 and 6). A seemingly intolerant hybrid at both locations was Hyperformer HS 97 with yields ranging from 5039.3-5811.2 kg/ha. Several hybrids were damaged by nicosulfuron herbicide. In particular, Pioneer Brand 3154 at Montgomery County exhibited top necrosis and retarded growth on about 20% of it's plants as a result of this herbicide application. This hybrid yielded poorly despite of it's low level of disease.

Table 3. Hybrid performance under heavy gray leaf spot infection in Montgomery County, Virginia, 1989.

Hybrid name	Disease Severity		Index(0-5)[1]	% Lodged	% H_2O	Yield[2]
	25JUL89	14AUG89	21SEP89	9OCT89	at harv.	kg/ha
Jacques 7910	1.25b-f[3]	3.22f-j	5.00a	5.9ef	21.6c-f	8340.2a
Hytest HT 650A	1.00efg	3.2g-j	4.99abc	7.1ef	21.1c-f	7580.9ab
Wilson EXP 1352	1.09def	3.20g-j	5.00a	8.0ef	22.3a-d	7543.2ab
Hytest HT 736	1.15c-f	3.28e-i	5.00a	6.6ef	21.5c-f	7499.3ab
Jacques 8280	1.03efg	3.17hij	5.00a	2.5f	23.0b-e	7235.7abc
Beck's 83X	0.98fg	3.23f-j	5.00a	5.1f	22.0b-e	7235.7abc
Pioneer Brand 3328	0.98fg	3.15ij	4.97d	11.4def	20.3def	7154.1a-d
Jacques 8210	1.05ef	3.40c-f	5.00a	8.9def	22.3a-d	7122.8a-e
Pioneer Brand 3352	1.53b-f	3.47cd	4.99c	15.0c-f	18.1g	7122.8a-e
Hytest X7809	1.13c-f	3.22f-j	4.99bc	39.5a-d	19.5fg	6834.1b-e
Beck's EXP 0200	0.67g	3.07j	4.97d	28.8a-f	21.1c-f	6815.3b-e
Wilson 1890	1.06def	3.28e-i	5.00a	22.9b-f	24.1a	6677.2b-e
Hytest HT 712	1.23b-f	3.30e-i	5.00a	19.4c-f	21.7b-e	6576.8b-e
Southern States 807	1.27b-f	3.25f-j	5.00a	19.4c-f	21.7b0e	6576.8b-e
Wilson EXP 1143	0.99efg	3.30e-i	5.00a	20.4b-f	20.6def	6319.5b-f
Pioneer Brand 3136	1.25b-f	3.38c-g	4.99ab	50.8ab	22.9abc	6319.5b-f
Pioneer Brand 3471	1.35b-e	3.53c	5.00a	18.8c-f	16.3h	6219.1b-f
Wilson EXP 7332	1.06def	3.30e-i	5.00a	32.7a-f	24.1a	6168.9b-f
Pioneer Brand 3192	1.48bc	3.30e-i	5.00a	27.1a-f	23.6a	6156.3b-f
Pioneer Brand 3140	1.28b-f	3.25f-j	4.99a	21.7b-f	21.5c-f	6099.8b-f
Hyperformer HS-97	0.98fg	3.22f-j	5.00a	22.9b-f	21.4c-f	5830.0c-g
Hyperformer X-8819	1.17b-f	3.27e-i	4.99abc	32.1a-f	21.4c-f	5786.1c-g
Jacques 9220	1.27b-f	3.70b	5.00a	23.5b-f	20.5def	5723.3d-g
Hyperformer HS-56	1.00efg	3.20g-j	4.99c	36.9a-c	22.3a-d	5660.6efg
Hyperformer HS-64	1.22b-f	3.25f-j	5.00a	31.4a-f	21.2c-f	4995.3fgh
Jacques 8250	1.15c-f	3.22f-j	5.00a	33.0a-f	21.5c-f	4926.3fgh
Southern States 844	1.15c-f	3.45cde	5.00a	44.8abc	21.8b-e	4675.3gh
Southern States 814	1.42bcd	3.38c-g	5.00a	33.2a-f	21.9b-e	4662.7gh
Pioneer Brand 3320	1.93a	3.95a	5.00a	9.4def	18.2g	4505.8gh
Hyperformer HS-889	1.20b-f	3.35d-h	5.00a	54.3a	20.7def	4167.0h
LSD(0.05)=	0.30	0.15	0.01	25.2	1.7	19.1
Standard Deviation=	0.214	0.104	4.469	17.80	1.2	13.53
CV=	18.13	3.15	0.09	77.73	5.67	13.48

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Yield expressed as kg/ha at a standard 15.5% moisture content.

[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 4. Hybrid performance under heavy gray leaf spot infection in Wythe County, Virginia, 1989.

Hybrid name	Disease	Severity	Index(0-5)[1]	% Lodged	% H_2O	Yield[2]
	27JUL89	14AUG89	21SEP89	11OCT89	at harv.	kg/ha
Jacques 8210	1.42b-f[3]	3.17efg	4.99a	55.6a-f	26.7a	6765.0a
Beck's 83X	1.30efg	3.15efg	4.97a	49.0b-f	23.9b-f	6376.0ab
Hytest HT 650A	1.30efg	3.20d-g	4.89a	60.1a-e	22.1f-i	6231.6abc
Beck's EXP 0200	1.02gh	3.05g	4.57b	47.7b-g	22.6e-i	6175.1abc
Jacques 7910	1.27efg	3.25def	5.00a	28.5efg	24.4b-e	6162.6abc
Pioneer Brand 3328	0.90h	3.10fg	4.92a	24.8efg	21.2hi	6143.8abc
Hyperformer X-8819	1.57b-e	3.32de	4.97a	83.6ab	24.0b-f	5905.3a-d
Pioneer Brand 3352	1.70a-d	3.38cd	4.97a	11.4g	21.7ghi	5880.2a-d
Jacques 8280	1.32d-g	3.15efg	4.98a	31.6efg	25.7ab	5867.6a-d
Hyperformer HS-56	1.10fgh	3.20d-g	4.94a	61.7a-e	24.1b-f	5346.8b-e
Pioneer Brand 3136	1.78ab	3.33de	4.98a	73.8abc	25.5abc	5309.1b-e
Hytest HT 736	1.50b-e	3.15efg	4.98a	38.8c-g	24.2b-e	5052.0c-f
Hytest HT 712	1.35c-g	3.22d-g	5.00a	68.6a-d	23.0e-h	4832.2d-g
Pioneer Brand 3471	2.02a	3.57b	5.00a	33.3d-g	17.5j	4662.7d-g
Southern States 807	1.55b-e	3.33de	4.96a	58.4a-e	23.0e-h	4493.3efg
Hytest X7809	1.58b-e	3.20d-g	4.99a	87.1a	22.0f-i	4480.7efg
Pioneer Brand 3192	1.72abc	3.53bc	4.99a	34.6d-g	25.7ab	4411.7efg
Pioneer Brand 3140	1.60b-e	3.38cd	4.99a	19.0fg	23.5c-g	4361.5efg
Southern States 814	1.50b-e	3.30de	4.99a	57.0a-e	24.5b-e	3909.7gh
Jacques 9220	1.63b-e	3.60ab	5.00a	39.5c-g	25.3a-d	3803.0fgh
Jacques 8250	1.33d-g	3.22d-g	4.99a	86.6a	23.3d-h	3595.9gh
Hyperformer HS-64	1.28efg	3.20d-g	5.00a	84.4ab	24.0b-f	3577.1gh
Pioneer Brand 3320	2.00a	3.75a	5.00a	28.6efg	20.7i	3106.4gh
Hyperformer HS-97	1.70a-d	3.30de	5.00a	72.3abc	23.7d-g	3031.1h
Hyperformer HS-889	1.40b-f	3.28def	4.99a	77.2ab	25.5abc	2780.1h
Southern States 844	1.73abc	3.50bc	5.00a	87.1a	25.5abc	2698.5h
LSD(0-5)=	0.32	0.15	0.08	30.8	1.80	17.3
Standard deviation=	0.225	0.109	5.462	21.764	1.27	12.24
CV=	15.14	3.30	1.10	40.41	5.39	15.98

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Yield expressed as kg/ha at a standard 15.5% moisture content.

[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 5. Hybrid performance under heavy gray leaf spot infection in Montgomery County, Virginia, 1990.

Hybrid name	Disease Severity		Index(0-5)[1]	% Lodged	% H_2O	Yield[2]
	8AUG90	30AUG90	20SEP90	29OCT90	at harv.	kg/ha
Pioneer Brand 3140	1.72e[3]	3.00fg	4.13d-g	3.6bc	16.6efg	8183.3a
Wilson 1852	1.95a-d	3.08efg	4.47abc	5.8bc	18.0bcd	7825.6ab
Pioneer Brand 3136	1.93bcd	3.07efg	4.32b-e	5.8c	18.1bcd	7618.5
Hytest HT 736	1.92bcd	3.00fg	4.35b-e	0.9c	22.0c-f	7235.7a-d
Hytest HT 650A	1.98abc	3.17cde	4.45a-d	1.3c	15.8fgh	7072.6a-d
Hytest HTX7809	1.90bcd	3.00fg	4.13d-g	2.1c	17.0def	7066.3a-d
Augusta P560	1.98abc	3.07efg	4.33b-f	2.2c	16.2efg	6915.7a-e
Hyperformer HS 9911	1.97abc	3.17cde	4.51abc	3.77bc	16.4efg	6696.0a-e
Augusta 505	1.98abc	2.97gh	4.00fg	1.3c	17.5cde	6677.2a-e
Augusta 614	2.00ab	3.20cd	4.53abc	3.8bc	18.8bc	6671.0a-e
Hyperformer HS 9773	1.95a-d	3.15cde	4.55ab	6.1bc	14.9h	6526.6b-e
Hyperformer HS 9663	2.03ab	3.10def	4.28b-g	1.9c	16.0fgh	6482.6b-e
Pioneer Brand 3192	2.10a	3.17cde	4.35b-e	1.9c	18.5abc	6470.1b-e
Wilson Demand 110	1.95a-d	3.25bc	4.73a	2.1c	15.5gh	6470.1b-e
Dekalb Dk 689	1.88b-e	2.61i	3.98g	4.7bc	19.7a	6438.7b-e
Pioneer Brand 3154	1.80de	2.88h	4.02efg	12.2a	18.2bcd	6407.3cde
Pioneer Brand 3352	1.83cde	2.97gh	4.20c-g	1.3c	16.0fgh	6087.3cde
Hyperformer HS 97	2.10a	3.35ab	4.74a	9.1ab	16.1fgh	5811.2de
Pioneer Brand 3320	2.10a	3.43a	4.76a	2.7c	45.5gh	5428.4e
Wilson EXP 3166	2.00ab	3.17cde	4.35b-e	3.9bc	16.9def	5321.7e
LSD (0.05)=	0.14	0.10	0.28	1.1	5.0	21.7
Standard deviation=	9.83	7.29	0.20	0.8	3.6	15.3
CV=	5.03	2.36	4.57	4.80	93.84	14.41

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Yield expressed as kg/ha at a standard 15.5% moisture content.

[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 6. Hybrid performance under heavy gray leaf spot infection in Wythe County, Virginia, 1990.

Hybrid name	Disease Severity		Index(0-5)[1]	% Lodged	% H_2O	Yield[2]
	8AUG90	30AUG90	20SEP90	29OCT90	at harv.	kg/ha
Pioneer Brand 3136	1.88a-e[3]	2.70bcd	4.43fg	15.4c-h	23.3abc	8948.9a
Pioneer Brand 3140	1.85a-e	2.85bc	4.75b-g	25.2a-g	20.3ghi	7436.5b
Helena 9663	1.83b-f	2.57bcd	4.71a-d	20.8b-h	20.5f-i	7348.7b
Pioneer Brand 3352	1.83b-f	2.45cde	4.40g	3.7h	20.0hi	7304.7b
Pioneer Brand 3154	1.40g	1.95f	4.05h	32.5a-d	24.5a	7285.9b
Wilson Demand 110	1.78b-f	2.98b	4.74abc	2.5h	20.9e-i	7267.1b
Wilson 1852	1.68ef	2.40c-f	4.48d-g	25.3a-g	23.7ab	7254.5b
Pioneer Brand 3192	1.97abc	2.67bcd	4.56b-g	5.9gh	22.7bcd	7210.6b
Augusta P560	1.80b-f	2.63bcd	4.55c-g	4.2h	21.2d-i	7166.7b
Hytest HT 650A	1.82b-e	2.50b-e	4.45efg	8.4e-h	21.5d-h	6677.2bc
Hytest HT 736	1.83b-f	2.65bcd	4.60b-g	6.9fgh	22.0c-f	6664.6bc
Augusta 614	1.83b-f	2.38c-f	4.61b-g	12.9d-h	22.4b-e	6614.4bc
Augusta 505	1.75c-f	2.08ef	4.55c-g	26.2a-f	21.9c-g	6325.8bcd
Dekalb DK 689	1.70def	2.40c-f	4.05h	38.9ab	22.3b-e	6244.2bcd
Wilson EXP 3166	1.58fg	2.63bcd	4.68a-e	44.9a	21.5d-h	6118.7bcd
Hytest X7809	2.03ab	2.32def	4.65a-f	26.8a-e	21.6d-h	6087.3bcd
Pioneer Brand 3320	2.10a	3.58a	4.86a	21.0b-h	21.0e-i	5867.6bcd
Hyperformer HS 97	1.98abc	2.97b	4.80ab	29.9a-d	20.4f-i	5039.3cd
Hyperformer HS 9911	2.10a	2.88bc	4.88a	35.0abc	20.9e-i	4876.1d
Hyperformer HS 9773	1.95a-d	3.00b	4.80ab	39.8ab	19.7i	4706.7d
LSD (0.05)=	0.22	0.43	0.20	16.9	1.4	23.6
Standard deviation=	0.15	0.30	0.14	11.9	0.9	16.7
CV=	8.42	11.48	3.12	55.96	4.484	15.84

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Yield expressed as kg/ha at a standard 15.5% moisture content.

[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 7. Hybrid performance under heavy gray leaf spot infection in Montgomery County, Virginia, 1991.

Hybrid name	Disease Severity		Index(0-5)[1]	% Lodged	% H_2O	Yield[2]
	12AUG91	3SEP91	20SEP91	10OCT91	at harv.	kg/ha
Pioneer Brand 3154	2.53f	4.10ef	4.90c	2.6ab	18.4ab	6407.3a
Pioneer Brand 3136	2.93bc	4.35bc	4.95b	0.4cd	17.5bc	6376.0a
Hytest HT X7510	2.70de	4.50b	5.00a	0.0d	15.1efg	6030.8ab
Pioneer Brand 3241	2.85cd	4.30cd	4.93bc	2.6ab	17.3c	5572.7abc
Dekalb DK 689	2.55f	4.03f	4.89d	2.0abc	18.7a	5365.6abc
Pioneer Brand 3140	2.72de	4.45b	4.96ab	3.7a	15.8d-g	5189.9abc
Pioneer Brand 3192	3.03ab	4.43bc	4.95b	1.3bcd	18.8a	4894.9abc
Augusta 613	3.10a	4.72a	5.00a	0.0a	15.4efg	4819.6abc
Hytest HT 744	3.07ab	4.72a	5.00a	0.0d	14.9fg	4769.4abc
Hytest HT 736	2.80cd	4.38bc	5.00a	0.9bcd	16.7cd	4725.5abc
Augusta 513	3.05ab	4.70a	4.99a	0.0d	15.1efg	4637.6abc
Pioneer Brand 3352	2.83cd	4.17de	4.85d	1.0bcd	16.1de	4330.1bc
Hytest HT X7728	2.78cd	4.47b	4.96ab	0.0d	15.3efg	4323.9bc
Dekalb DK 689	2.55f	4.03f	4.86d	2.0abc	18.7a	5365.6abc
Pioneer Brand 3245	3.05ab	4.70a	5.00a	0.4cd	15.2efg	4123.0bc
Pioneer Brand X0-813	3.00ab	4.47b	4.96ab	0.9bcd	17.2c	4060.3bc
Pioneer Brand 3320	3.15a	4.75a	5.00a	0.8bcd	14.6g	3821.8c
LSD (0.05)=	0.14	0.13	0.03	1.63	1.0	26.2
Standard deviation=	0.0958	0.0915	0.0235	1.1422	0.7039	18.3610
CV=	3.34	2.06	0.47	117.15	4.31	23.40

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Yield expressed as kg/ha at a standard 15.5% moisture content.

[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 8. Hybrid performance under heavy gray leaf spot infection in Wythe county, Virginia, 1991.

Hybrid name	Disease Severity Index(0-5)[1]		% Lodged	% H_2O	Yield[2]
	27AUG91	17SEP91	11NOV91	18NOV91	kg/ha
Hytest HT X7510	2.70c-f	4.33bcd	0.4a	21.2de	9018.0a
Pioneer Brand 3154	2.35f	3.97g	0.8a	25.7ab	8936.4ab
Augusta 613	2.65def	4.40b	0.4a	22.6cd	8810.9ab
Pioneer Brand 3241	3.08abc	4.20c-f	1.2a	21.8de	8792.1abc
Pioneer Brand 3136	3.02a-d	4.22c-f	1.1a	24.3bc	8572.0a-d
Hytest HT 736	2.75cde	4.18def	0.4a	25.9a-d	8522.2a-d
Pioneer Brand 3192	2.95a-d	4.28b-e	0.8a	27.0a	8503.4a-d
Pioneer Brand X0-813	3.28a	4.43b	0.0a	22.0cde	8465.7a-d
Pioneer Brand 3140	2.85b-e	4.20c-f	0.0a	22.2cd	8403.0a-d
Augusta 513	2.95a-d	4.43b	0.5a	22.6cd	7944.8a-e
Pioneer Brand 3245	3.15ab	4.35bc	0.4a	19.5e	7913.5a-e
Dekalb DK 649	2.67def	4.10fg	0.0a	25.4ab	7907.2a-e
Hytest HT X7728	2.67def	4.07fg	0.0a	25.9ab	7819.3b-e
Dekalb DK 689	2.53ef	3.97g	0.8a	25.8ab	7656.2cde
Pioneer Brand 3352	2.75cde	4.15ef	0.0a	21.1de	7643.6de
Hytest HT 744	3.00a-d	4.20c-f	0.0a	27.2a	7486.7de
Pioneer Brand 3320	3.20ab	4.57a	0.3a	20.5de	6890.6e
LSD (0.05)=	0.33	0.15	1.13	2.2	15.3
Standard deviation=	0.231	0.104	0.787	1.520	10.680
CV=	8.08	2.46	190.49	6.45	8.18

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Yield expressed as kg/ha at a standard 15.5% moisture content.

[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

In 1991, more resistant hybrids, Pioneer Brand 3154, 3136, and Hytest HT X7510, yielded in the top 20% all of hybrids tested and showed low DSI's. Significant differences of all hybrids used in 1991 are charted on Tables 7 and 8.

To determine sources of DSI interaction between years and location, six hybrids common to each were statistically analyzed by GLM. Common hybrids were Pioneer Brand 3136, Pioneer Brand 3140, Pioneer Brand 3352, Pioneer Brand 3192, Hytest HT 736, and Pioneer Brand 3320. The ANOVA of gray leaf spot DSI (Table 1) indicated these ratings differed significantly with a $P \leq 0.0001$, because of location, year, hybrid, and rating date. A significant interaction between year and location showed that environment may be affecting the epidemiology of GLS. The F value of 263.3 was highly significant at $P \leq 0.0001$. Comparatively, the same hybrid responded differently to blighting at both locations in each year. At Montgomery County in 1991, the mean yield was $\leq 24.6\%$ lower than in previous years. These results could reflect the hybrid's response to dry and hot weather conditions and the effects of these on corn performance and GLS development.

The ANOVA for grain yield in kg/ha (Table 2) indicated that location and year effects are highly significant as expected due to varying disease pressure and environmental conditions. This was confirmed by a significant interaction ($P \leq 0.0001$) between location and year. Hybrids were also significantly different ($P \leq 0.0001$). The same hybrid yielded differently each year at both locations as indicated by a significant F value of 3.03.

Comparison of the tolerant (Pioneer Brand 3136) versus the susceptible (Pioneer Brand 3320) hybrid's DSI with total rainfall for each rating period over three years at the Montgomery County showed that 12 cm of rain fell prior to the July disease rating and rains continued throughout the growing season, totaling 69.5 cm (Figures C.1, D.1), in 1989 (Figure 1). Blighting was more severe by the mid-late August rating than in other years. Short intervals of temperatures ranging from 25 C in late-May to 8.7 C in late-August occurred throughout the growing season (Anonymous, 1989) seemed to have little affect on GLS development. In 1990, total rainfall equalled 65.9 cm, however the months of June and September were extremely dry with 8.3 and 4.6 cm, respectively (Figures C.1, D.1). During months of June through August, temperatures of 22 C or greater (Anonymous, 1990) produced droughty conditions with many of the hybrids showing rolled leaves by mid-day. Compared to 1989, these dry weather conditions appeared to severely retard disease development. These observations could be explained by the observations of Rupe *et al.* (1982) who found that moisture conditions effect spore survival, since short periods of dryness resulted in death of *C. zeae-maydis* spores on glass slides. In 1991, total rainfall was ≥ 2.3 times less than 1989 and 1990 (Anonymous, 1989, 1990, and 1991). Fortunately, rainfall early in the season was conducive to corn and GLS development. However, this was followed by four months of dry hot weather with averaged weekly temperatures ranging from 23.7-30 C (Figures A.3, B. 1) which may explain the retarded GLS disease development. In late-August to early September rainfall totalled 4.78 cm (Figures C.1, D.1) with high averaged

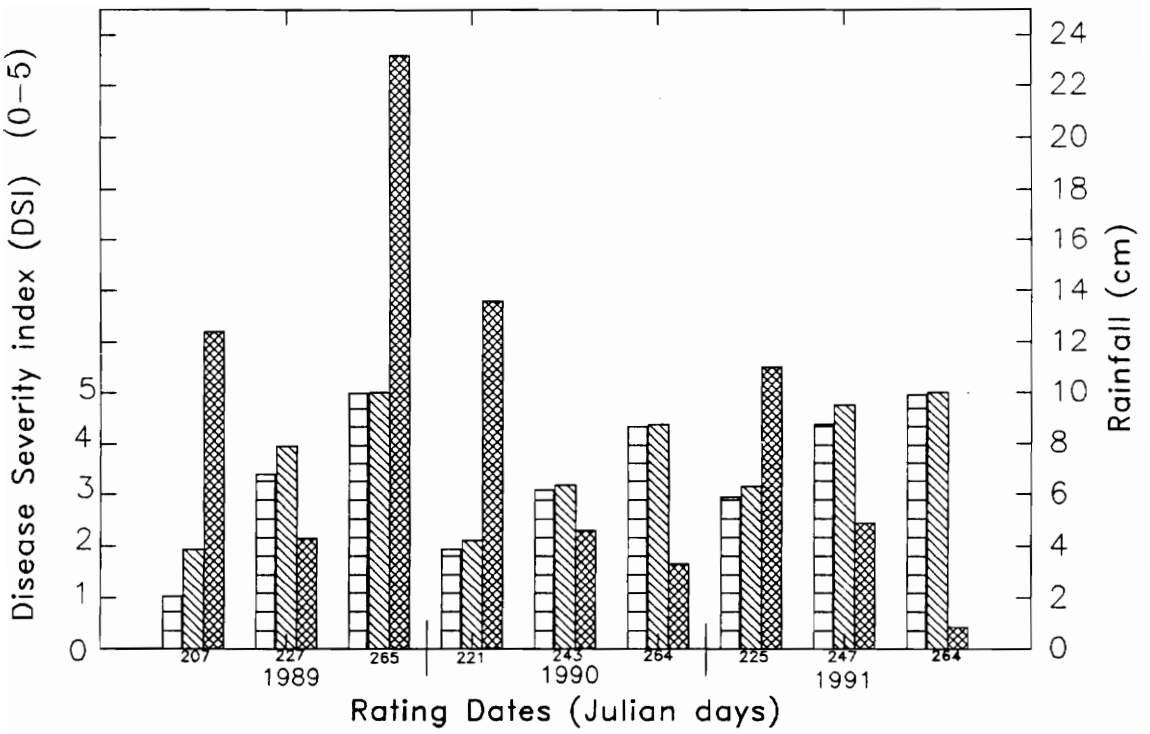

Figure 1. Comparison of gray leaf spot DSI for a tolerant hybrid, Pioneer Brand 3136 (horizontal lines) and a susceptible hybrid, Pioneer Brand 3320 (diagonal lines) with rainfall obtained between ratings (crosshatch) at Montgomery County, Virginia.

weekly temperatures around 25 C (Figures A.3, B.2) and disease increased rapidly. Under these moist conditions, *C. zeae-maydis* spore germination could have increased since its optimum growth temperature is 25C (Rupe, *et al.*, 1982).

Despite adverse weather conditions, Pioneer Brand 3136 consistantly scored a lower DSI than the susceptible Pioneer Brand 3320, as did the other six hybrids over three years (Table 9). The retarded disease development and dry hot weather patterns were correlated to a reduction in yields (Table 9). Yield, however, for Pioneer Brand 3136 was approximately 1.5 times greater than that of Pioneer Brand 3320.

Table 9. Hybrid performance under heavy gray leaf spot infection in Montgomery and Wythe Counties, Virginia, over three years.

		Disease Severity Index(0-5)[1]			
Hybrid Name	Location	late-Jul –mid-Aug	mid-Aug –Sept	mid-late Sept	Yield[2] kg/ha
Pioneer Brand 3136	Montgomery	2.02[3]	3.60	4.76	6770.7
	Wythe	2.23	3.42	4.70	7609.11
Hytest HT 736	Montgomery	1.96	3.55	4.78	6487.7
	Wythe	2.02	3.33	4.74	6746.8
Pioneer Brand 3140	Montgomery	1.91	3.57	4.69	6490.2
	Wythe	2.10	3.47	4.80	6735.6
Pioneer Brand 3352	Montgomery	2.06	3.54	4.72	5845.1
	Wythe	2.09	3.33	4.65	6943.3
Pioneer Brand 3192	Montgomery	2.20	3.63	4.80	5839.4
	Wythe	2.22	3.49	4.78	6707.9
Pioneer Brand 3320	Montgomery	2.39	4.04	4.92	4585.5
	Wythe	2.43	3.97	4.79	5287.8

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Yield expressed as kg/ha at a standard 15.5% moisture content.

[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

When the DSI's for both resistant and susceptible hybrids were compared with rainfall at the Wythe County location, in 1989, DSI's of Pioneer Brand 3136 and 3320 were similar to those obtained at Montgomery County (Figure 2). In 1989 and 1990, averaged weekly temperatures were also similar (Figure A.1, A.2), however, Wythe County recieved ≤ 9.13 cm more total rainfall (Figure C.1, D.1). In 1990, 3.35 and 8.89 cm of total rainfall, in dry months of June and July, seemed to slow the GLS epidemic in Montgomery County (Figure C.1, D.2). In 1991, however, Wythe County suffered much drier conditions than the Montgomery County location. Adequate rainfall in May was available for early crop growth, however, locations only recieved 1.11, 3.84, 5.26, and 0.56 cm of rain, in June, July, August, September, and October, respectively (Figure C.3, D.2). Temperatures did not seem to be a large factor in GLS development over all years, however it is noted that the weather station in Wythe County was approximately 24 km from the plot location.

Greater rainfall would be expected to increase corn yields and GLS development, however, Pioneer Brand 3136 and Pioneer Brand 3320 for Montgomery County had lower yields and higher GLS disease severity with less rainfall, than the Wythe County location where higher yields and lower blighting occurred (Tables 3-8). This pattern was consistant over all three years and for all six common hybrids (Table 9). In comparing soils at both sites, pH was equivalent and nutrients properly managed, however, Montgomery County's deep alluvial soil would have presumably promoted better crop vigor than the shallow soil of the Wythe County location. Although, the Montgomery

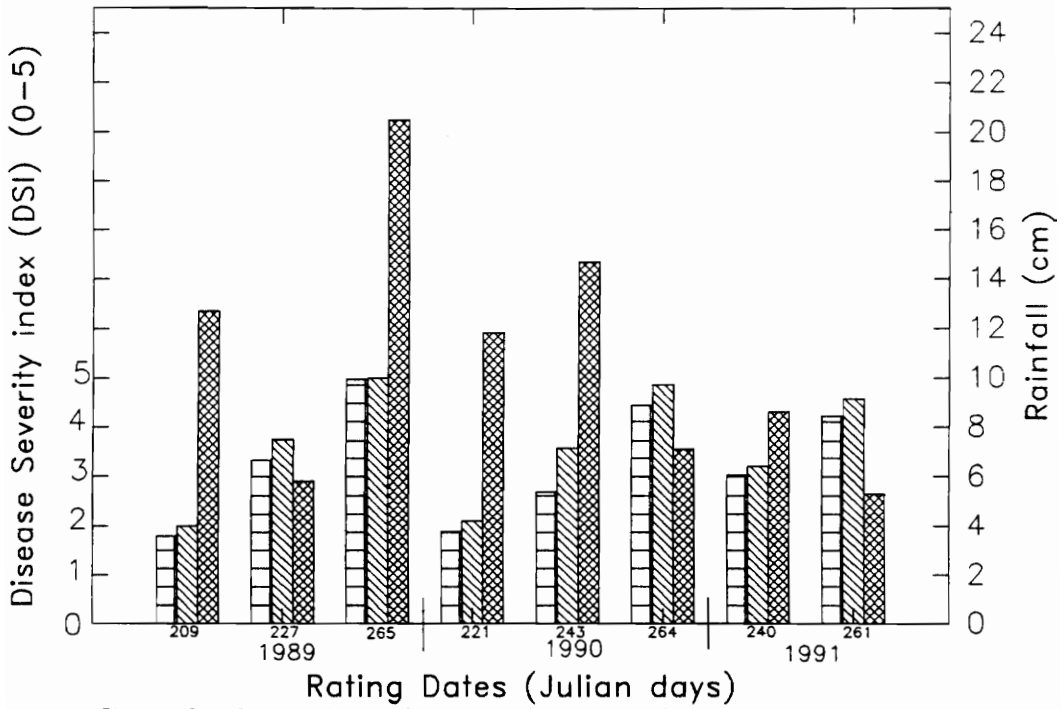

Figure 2. Comparison of gray leaf spot DSI for a tolerant hybrid, Pioneer Brand 3136 (horizontal lines) and a susceptible hybrid, Pioneer Brand 3320 (Diagonal lines) with rainfall obtained between ratings (crosshatch) at Wythe County, Virginia.

County location had a greater incidence of overcast skies and periods of prolonged leaf wetness than the Wythe location, which seemed to intensify GLS disease development and reduced grain yield. The rainfall at Wythe County tended to increase hybrid vigor, decrease disease severity, and promoted higher yields, contrary to the Montgomery County location. Overcast skies, high relative humidity and prolonged leaf wetness have been identified as factors that intensify the GLS disease epidemic (Beckman, *et al.*, 1981), since *C. zeae-maydis* germination and germ tube growth is maximized (Rupe, *et al.*, 1982).

Despite weather patterns over years and locations, those six common hybrid's DSI's from both locations were regressed individually against yield. Yields ranged from 7609.1 kg/ha for the most resistant hybrid (Wythe County) to 4585.5 kg/ha for the most susceptible hybrid (Montgomery County) (Table 9). A 30% correlation for blighting to yield was indicated at the late-July to mid-August ratings. The percent of yield loss that could be attributed to blighting increased to 77% in mid-late August (Figure 3), and then dropped to 42% at the mid-September rating.

In a comparison of the yield and blighting of common hybrids in late-July to early August at Montgomery County, an R^2 value of 0.84 between blighting and yield early in the growing season (Figure 4). By mid-late August, the correlation was 76% (Figure 5) and then decreased to an R^2 value of 0.50 by mid-September (Figure 6). The mid-season DSI usually has the greatest reliablilty in predicting the affect of blighting on hybrid yields, since photosynthate needed for grain fill is most important at mid-season and plant

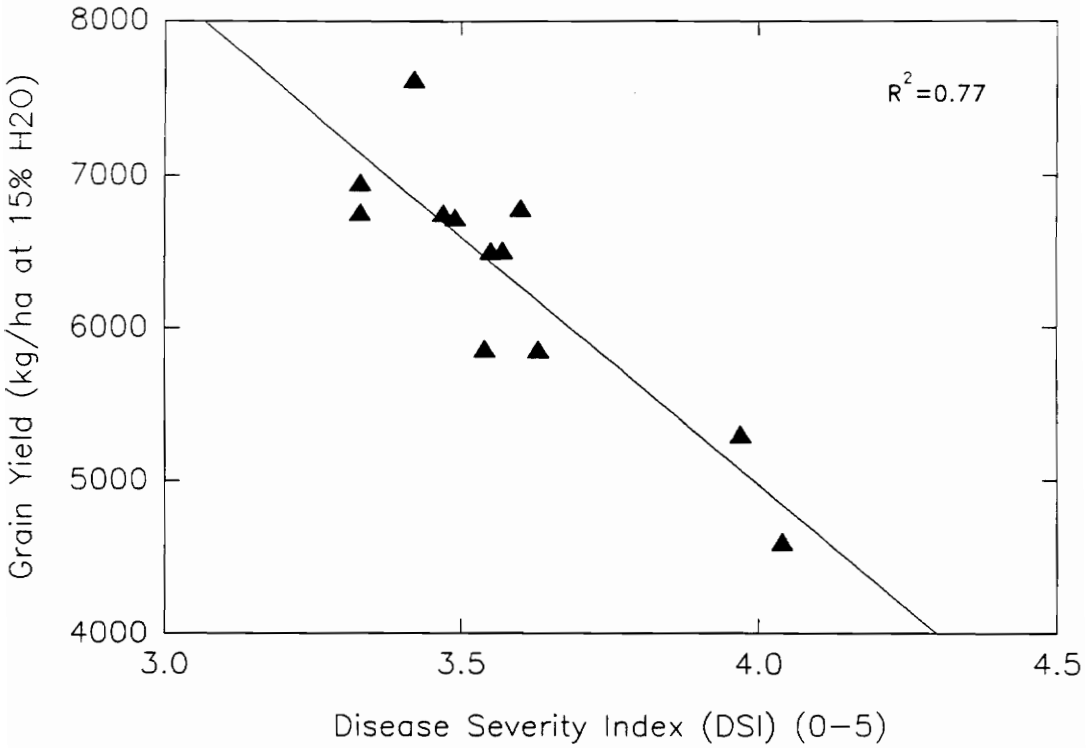

Figure 3. Comparison of grain yield and DSI in mid–late August at Montgomery & Wythe Counties, Virginia for six hybrids (as denoted by triangles) over three years.

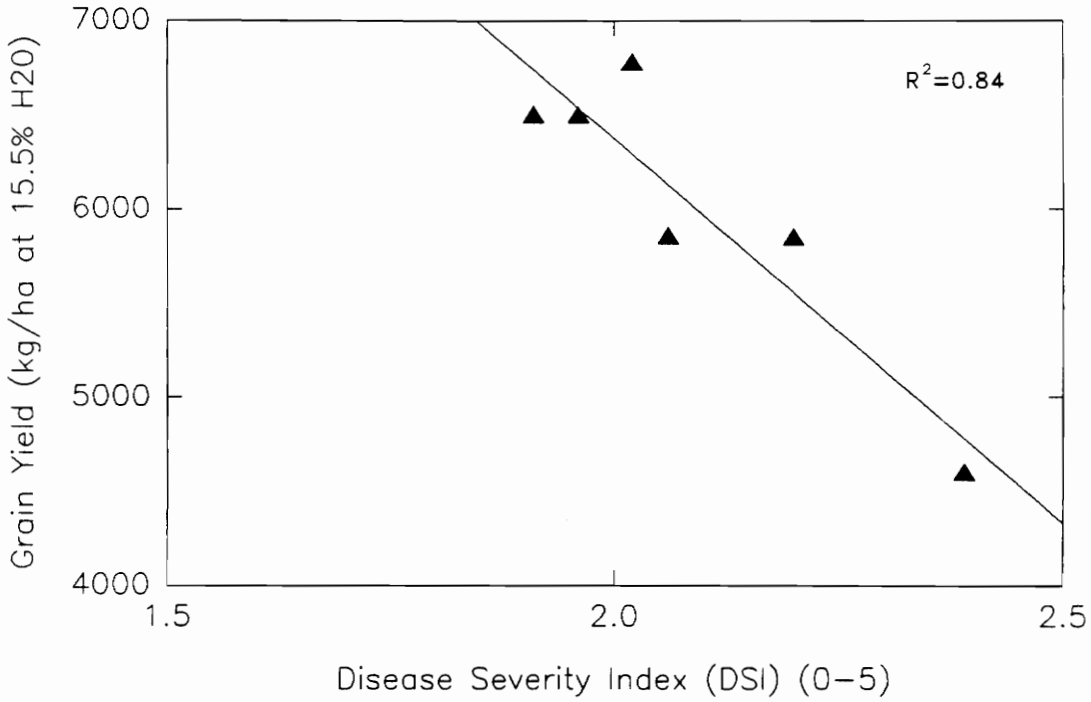

Figure 4. Comparison of grain yield and DSI in late–July to early August at Montgomery County, Virginia for six common hybrids (as denoted by triangles) over three years.

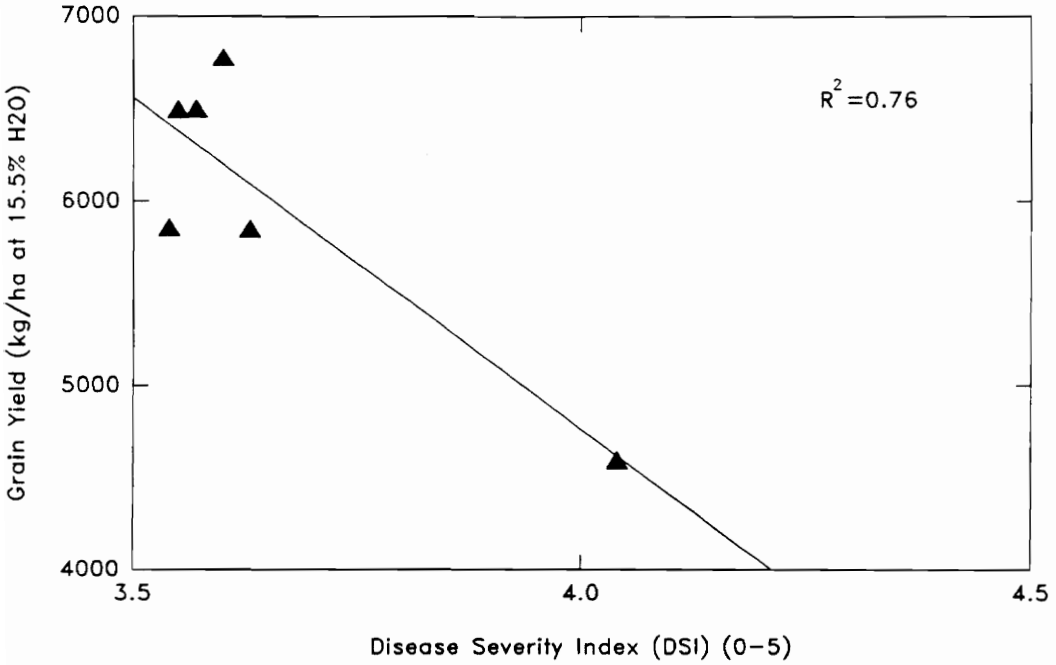

Figure 5. Comparison of grain yield and DSI in mid-late August at Montgomery County, Virginia for six common hybrids (as denoted by triangles) over three years.

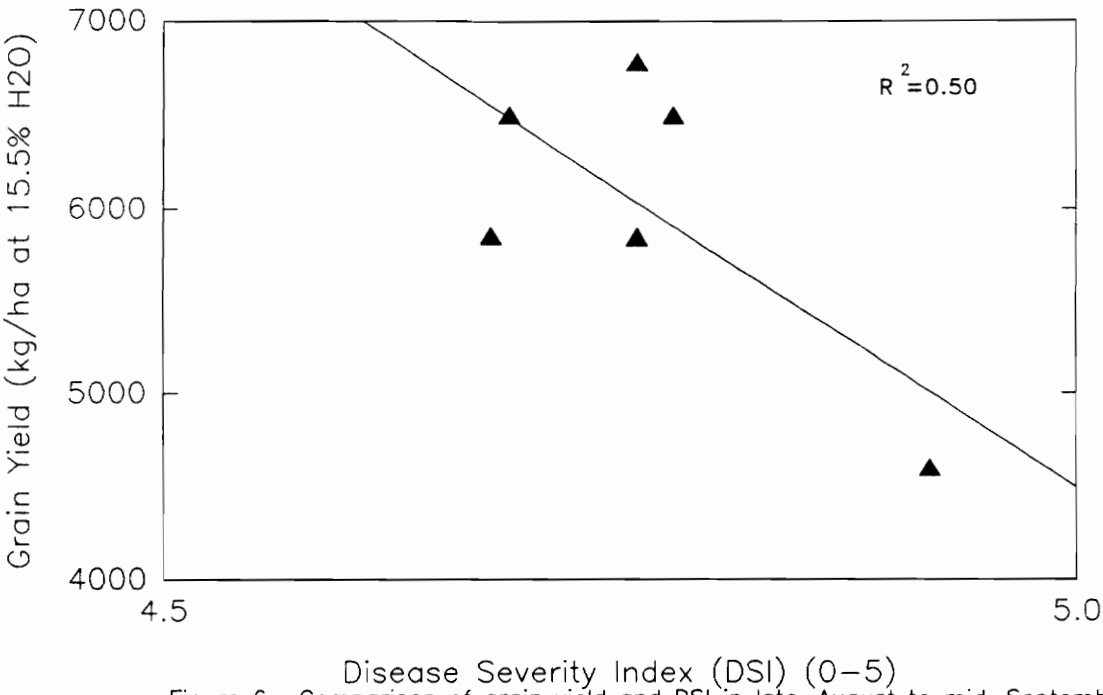

Figure 6. Comparison of grain yield and DSI in late-August to mid-September at Montgomery County, Virginia for six common hybrids (as denoted by triangles) over three years.

senescence occurs late in the season, that may interfere with visual ratings for disease. This is confirmed by observations of Stromberg and Donahue (1986). Regression was performed in the same manner for Wythe County, where the early-, mid-, and late ratings showed a 37, 75, and 35 percent correlation between blight and grain yield (Figure 7).

Regression analyses for blighting verses grain yield at Montgomery County using the common hybrids indicated that GLS blighting alone did not cause all yield loss. Blighting at the Montgomery County location caused greater portion of yield loss than at the Wythe County location. This infers that high temperature was not a limiting factor for the epidemic of GLS disease, however, high temperatures and lack of overcast skies and prolonged leaf wetness do decrease disease development. Beckman, *et al.* (1982) similarly reported that high temperatures and low levels of rainfall does not limit GLS disease, however, conditions conducive for disease development, *ie.*, high relative humidity and overcast skies are important.

Considering the many variables associated with field experiments, such as site location, rainfall plus temperature interaction, and year, this study demonstrated that this method of evaluating hybrids for resistance (performance under heavy disease pressure) was useful to predict the effect of blighting on yield, regardless of the location. DSI's and yield rankings are important to seed companies and farmers because of their ability to detect differences in performance between hybrids. Using more resistant hybrids for protection against GLS disease epidemics is essential in areas where conventional and/or no-tillage, continuous corn practices are common and GLS occurs regularly. Using more

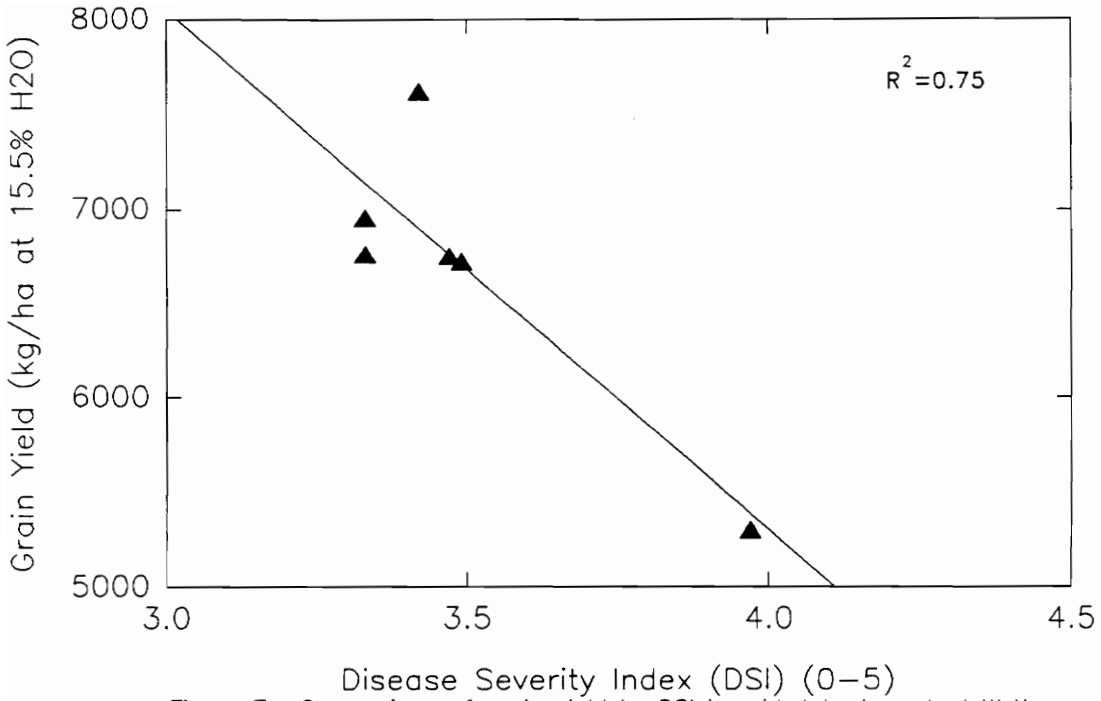

Figure 7. Comparison of grain yield by DSI in mid–late August at Wythe County, Virginia for six common hybrids (as denoted by triangles) over three years.

resistant hybrids will increase yields and may aid, in part, in reducing inoculum carried over to the next year.

ACKNOWLEDGEMENTS This research was funded in part by the Virginia Corn Board and the following companies; Augusta Seed Corp., Beck's Superior Hybrids, Dekalb-PIizer Benetics, Garst Seed Co., Helena Chemical Co., Jacques Seed Co., Northrup King, Co., Pioneer Hi-bred International, Inc., Stanford Seed Co., Southern States, Sungene Technologies Corp., and Wilson Hybrids, Inc. Special thanks to Lloyd Flinchum for his technical assistance establishing and maintaining plots and additional thanks to Harold Leedy , Wythe County for the use of his corn farmland.

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Effect of Foliar Fungicides on Gray Leaf Spot Disease and Yield of Corn

by

M.R. Carter

ABSTRACT

Fungicides applied foliarly were evaluated for control of gray leaf spot (GLS) disease of corn for three years. Assessment of leaf blighting (Disease Severity Index [DSI]), lodging, grain moisture at harvest, grain yield, and kernel weight were determined for the hybrid, Pioneer Brand 3320, treated and nontreated with fungicides. All fungicide treatments, with the exception of mancozeb, provided significant ($P \leq 0.0001$) disease control over the nontreated check in all years. Fungicide applications significantly increased yields by 1.3-2.2 times and reduced blight, over the nontreated check depending on year and fungicide. Common fungicide applications combined over all years had DSI's that averaged from 0.7-4.8 by early to mid-September, depending on the treatment. Benomyl at 2 or 4 applications was the most effective followed by propiconazole and terbutrazole at 2 applications. Analysis of yield for common fungicides with respect to blighting showed as much as 93% of the variation in yield attributal to blight severity. Kernel weights obtained 2 of 3 years from treated plots increased by 7-26% over the nontreated. Variation in yield was attributed to a decrease in kernel weight by 57-92%, in 1990 and 91, respectively. Use of fungicides was positively correlated with increased grain yield, moisture at harvest, and kernel weight.

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Gray leaf spot (GLS) disease of corn (*Zea mays* L.) is caused by the fungus, *Cercospora zeae-maydis* Tehon and Daniels. GLS has become increasingly important to corn production not only in the mid-Atlantic region but also in the areas of Ohio, Indiana, Illinois, Iowa, eastern Kentucky, Tennessee and northeastern Missouri. Since *C. zeae-maydis* overwinters on corn debris (Payne and Waldron, 1983) and since, conservative tillage practices have increased over the decade, this increase in distribution and severity has been associated with minimum tillage practices, particularly in combination with continuous corn production. In addition, microclimates favorable to disease development can be produced under center-pivot irrigation systems in Kansas and Nebraska where GLS has been reported (Personal communication, Smith, Dekalb Genetics). GLS has also been reported in Mexico, Central and South America, and Trinidad (Latteral and Rossi, 1983).

The most severe infestations of GLS are observed in areas where extended morning and evening dews, and lingering fogs result in prolonged periods of high relative humidity and leaf wetness (Beckman and Payne, 1982, 1983); but the disease is not exclusively restricted to these areas when abundant initial inoculum is present (Latterell and Rossi, 1983). Rupe (1982) determined the influence of environment on survival of conidia and infection of *C. zeae-maydis* in Kentucky and found that high numbers of conidia are released under conditions of high relative humidity and extended leaf wetness.

Losses caused by GLS disease are due to premature loss of photosynthetic tissue. Blighting begins on the lower leaves of the corn plant, then progresses acropetally and,

when severe, causes a significant reduction in photosynthate required for grain fill. Depending on severity, early initiation and development, and degree of stalk lodging, losses in grain yield may range from 313.8 to 3137.8 kg/ha (Stromberg, 1990a, 1990b; Stromberg and Carter, 1991a, 1991b).

Measures to control gray leaf spot have included the elimination of infested debris from the previous corn crop by moldboard plowing or the reduction of inoculum by rotation of corn to a field previously not cropped to corn. Use of more tolerant or resistant hybrids limits disease development and minimizes losses (Stromberg and Donahue, 1986).

Benzimidazole fungicide derivatives have been shown to be effective against *C. beticola* Sacc., the causal agent of a leaf spot disease in sugarbeet (*Beta vulgaris* L.) (Schneider *et al.*, 1970; Solel, 1970). Their systemic activity has surpassed the activity of protectant-type fungicides used in sugarbeet (Smith and Martin, 1978). Ayers *et al.*, (1984) reported that protectant fungicide mancozeb (*sic* zinc-maneb) sprayed for control of GLS on corn increased yields by 20%. Similar studies were performed by Hilty *et al.*, (1978). Other systemic fungicides, such as triazoles, have shown blight reduction and yield increase for the control of foliar diseases of corn. Propiconazole and terbutrazole used to control northern leaf blight (NBL) applied twice during the growing season reduced blighting and increased grain yield (Lipps and Johnston, 1990). White (1988) also reported that propiconazole reduced blighting caused by NLB by 47.6% over the nonsprayed check.

The application of foliar fungicides is not recommended as economically practical means of control in cash grain corn production for Virginia. Fungicide application, however, may be economically profitable and justified for seed corn production fields in some areas of the midwest. Grain yield reduction caused by GLS disease has been previously shown by use of foliar fungicides to reduce blighting for a single high yielding, but susceptible hybrid (Stromberg, 1990c; Stromberg and Carter, 1991c; Carter and Stromberg, 1992).

The objectives of this study were 1) to critically evaluate the efficacy of foliar fungicides to control gray leaf spot on an adapted high yielding corn hybrid, and 2) to provide a continuum of blighting levels throughout the growing season to document grain losses associated with blighting.

MATERIAL AND METHODS

Foliar fungicides were evaluated for control of gray leaf spot disease of corn for three years at the University's Whitehorne-Kentland Plantation Experimental Farm in Montgomery Co., VA. This site is located adjacent to the New River and consistently exhibits high humidity and morning fogs. These conditions favor gray leaf spot disease development. Three years before this study was conducted, *C. zeae-maydis* was introduced to the site by inoculation of susceptible spreader rows with an isolate collected in Shenandoah County, Virginia. High levels of inoculum have been maintained by continuous no-tillage corn production. Prior to planting, atrazine (1.48 kg ai/ha) and metolachlor (1.86 kg ai/ha) were applied to the soil surface for weed control.

Nicosulfuron (0.035 kg ai/ha) was applied postemergence at approximately the 10th leaf stage for control of johnsongrass. A complete commercial fertilizer (47-23-30 NPK) was applied at 369.9 kg/ha, with the exception of the Wythe County site in 1989 (493.2 kg/ha) as prescribed by soil tests.

The experimental design was a randomized complete block replicated four times with plots consisting of four 7.6 m rows spaced 0.76 m apart. Plots were seeded with a susceptible, high yielding hybrid, Pioneer Brand 3320 on 17, 8, and 23 of May for 1989, 90, and 91, respectively at a rate of 52800 seeds/ha. The middle two rows of each plot unit were treated with fungicide applied, with a CO_2-pressured backpack sprayer, two and four times during the growing season at 14-21 day intervals and evaluated against a nontreated control. The following fungicides were applied in each of three years, two of three or one of three years; propiconazole (Tilt 3.6E) (Ciba-Geigy, Greensboro, NC) at 126 g ai/ha, terbutrazole (Folicur 3.6F) (Miles Inc, Kansas City, MO) at 126 g ai/ha plus X-77 at 0.125% v/v, and benomyl (Benlate 50DF) (E.I. DuPont de Nemours Co., Wilmington, DE) at 560 g ai/ha; Dupont experimental (DPX-H6573-241 25EC) (E.I. DuPont de Nemours Co., Wilmington, DE) 126 g ai/ha, Rohm and Haas experimental (RH-7592 2.0F) (Rahm and Haas, Philadelphia, PA) 126 g ai/ha; and mancozeb (Manzate 200DF) (E.I. DuPont de Nemours Co., Wilmington, DE) 1.86 kg ai/ha, respectively. Spray solutions were applied at a volume of 252 l/ha with a single TeeJet@ D2-25 hollow cone nozzle at 275 KPa. Spray solutions were applied to cover both upper and lower leave of treated plants.

The middle two rows of each plot were scored five times during the growing season for leaf blighting. A Disease Severity Index (DSI) ranging from 0-5 was used for this assessment as described by Roane *et al.* (1974) and expanded by Hilty *et al.* (1979). For this index, 0 = no symptoms, 1 = trace of lesions below the ear but only a trace of lesions above the ear, 2 = many lesions below the ear with a trace above the ear, 3 = severe lesion development below the ear and some lesions on leaves above the ear, 4 = all leaves show severe lesion development but green tissue is still visible, and 5 = all leaves are dead. The middle two rows were also scored for percentage lodging (number of plants lodged below the ear), grain moisture at harvest, grain yield, and 500 kernel weight. Percentage leaf area blighted, assessed by scoring five randomly selected ear leaves from each replication, was also determined on a blighting scale (0-60), similar to that of the stripe rust of wheat model illustrated by the Distrain program (Tomerlin and Howell, 1988), one of three years. Plots were mechanically harvested with a Gleaner E Combine modified for small plot use.

Percentage lodging, grain moisture, and grain yield expressed at a standard 15.5% moisture, were determined for each treatment in each growing season and significant differences were assessed by Duncans Multiple Range Test ($P \leq 0.05$). Analysis of variance (ANOVA) was completed for each growing season separately. Six fungicide treatments and a water control common to all years were combined for statisical analysis. Dependent variables, *ie.*, grain yield, lodging and grain moisture were analyzed with ANOVA using this General Linear Model (GLM):

Dependent variable= $\mu + \beta_j + \gamma_k + \Delta_l + (\beta\gamma)_{jk} + e_{jklm}$;

where j = 1,2,3 years, k = 0,1,2,3 fungicide treatments (0 = water alone), l = 1,2 number of applications, m = 1,2,3,4 blocks. The dependent variable, disease severity, was similarly analyzed by the following GLM:

Dependent variable= $\mu + \beta_j + \gamma_k + \Delta_l + (\beta\gamma)_{jk} + (\gamma\Delta)_{kl} + \delta_{jklm} + e_{jklmr}$;

where j = 1,2,3 year, k = 0,...7 fungicide treatments, l = 1,2 number of applications, m = 1,2,3,4 rating dates, r = 1,2,3,4 blocks. The effect of GLS on lodging, grain moisture, grain yield, 500 kernel weight, and percentage leaf area was estimated by the Linear Regression Model (LRM):

$$Y = B_o + B_i X_1 + E_i;$$

where Y is the response variable, B_o is the intercept, B_i is the slope of the regression line, X_1 is the regressor variable, and E_i is the extraneous variable.

RESULTS AND DISCUSSION

Climatic conditions varied substantially during years 1989, 90, and 91 and had a considerable affect on GLS development. These conditions ranged from a rather wet season in 1989 to a period of drouth from July to September, in 1991. In 1990, rainfall was sufficient for hybrid growth early in the season, but high temperatures and only moderate rainfall in June through August, retarded disease development. Combined analysis of variance for lodging, gray leaf spot ratings and yields resulted in a significant difference ($P \leq 0.0001$) among years (Tables 1, 2, and 3). Each year was analyzed separately because of variablility.

Table 1. Combined analysis of variance of percentage lodged for fungicide evaluations in 1989, 90, and 91.

Source of variation	df	Mean square	F value	P value
Year	2	1153.59	75.39	0.0001
Fungicide	3	10.67	0.70	0.5570
Number of Applications	1	3.07	0.20	0.6556
Block	3	31.09	2.03	0.1181
Year * Fungicide	6	7.88	0.51	0.7950
Error	68	15.30		
R^2=0.72				
CV=78.0				

Table 2. Combined analysis of variance of gray leaf spot rating for fungicide evaluations in 1989, 90, and 91.

Source of variation	df	Mean square	F value	P value
Year	2	35.88	150.01	0.0001
Fungicide	3	42.59	178.08	0.0001
Number of applications	1	17.33	72.44	0.0001
Rating date	3	148.97	622.76	0.0001
Block	3	0.21	0.88	0.4530
Rating * Block	9	0.09	0.42	0.9256
Year * Rating	6	5.25	21.95	0.0001
Error	346	0.24		
R^2=0.90				
CV=16.9				

Table 3. Combined analysis of variance of grain yield (kg/ha) for fungicide evaluations in 1989, 90, and 91.

Source of variation	df	Mean square	F value	P value
Year	2	6934.59	41.18	0.0001
Fungicide	3	7219.86	42.87	0.0001
Number of applications	1	323.59	17.77	0.1702
Block	3	331.34	1.97	0.1271
Year * Fungicide	6	559.23	3.32	0.0063
Error	68	168.04		
R^2=0.80				
CV=13.9				

GLS lesion development was apparent on lower leaves as early as June and progressed up to the ear leaf by late-July. The first fungicide applications were applied when blighting reached a DSI of ≤ 0.7. Despite variation in weather conditions over years, differences in blighting among fungicide treatments were usually detected by early milk stage (mid-late August). Much earlier symptom expression for GLS occurred in 1989 than in 1990.

All fungicides, except mancozeb with four applications, provided significant ($P \leq 0.05$) control of GLS of corn as compared to the nontreated check in all experiments conducted in 1989, 90, and 91 (Tables 4-6). In mid-late August ratings (Julian dates 225-243), benomyl with four applications reduced blighting, as indicated by low DSI ratings (ranging from 0.7 to 3.1), depending on the year. Application of fungicides resulted in significant ($P \leq 0.05$) increases in grain yields, ranging from 1.3 (mancozeb with two applications) to 2.2 times (benomyl with four applications) greater than the nontreated check. Mancozeb was removed from this study since blight control was not effective. Variance in yields between years can be attributed in part to hurricane Hugo which resulted in significantly more lodged plants (Table 1) that interferred with mechanical harvesting in 1989. Correlations of less than 1% between grain yields and lodging resulted in 1990 and 1991 (Tables 4-6).

Table 4. Fungicides for control of gray leaf spot (GLS) of corn in 1989.

Fungicide	Rate	no. of[0]	Disease Severity Index (DSI) (0-5)[1]				%	%	Yield
	g ai/ha	applic	207[2]	227	252	265	lodged	H_2O	kg/ha[3]
Nontreated		0	1.78a[4]	4.00a	4.86a	5.00a	10.9a	21.05ab	4405.4d
Propiconazole	126	2	1.08d	3.22de	4.15bc	5.00a	11.7a	20.88ab	6182.7bc
Propiconazole	126	4	1.15d	3.20de	3.88c	4.79b	12.9a	20.25ab	6255.5ab
Terbutrazole	126	2	1.10d	3.45bc	3.60c	4.38b	12.9a	21.13ab	5903.4bc
Terbutrazole	126	4	1.13d	3.37bc	3.53cd	4.13bc	11.5a	21.13a	6554.0bc
Benomyl	560	2	1.18cd	3.30cd	4.18bc	4.97a	10.5a	20.80ab	6142.5bc
Benomyl	560	4	1.15d	3.10e	3.50d	4.38c	11.8a	22.05a	7228.2a
Mancozeb	1860	2	1.30c	3.40bc	4.82a	5.00a	9.7a	19.97ab	5558.9bc
Mancozeb	1860	4	1.45b	3.48b	4.80a	5.00a	13.2a	19.53ab	5103.3cd
LSD (0.05) =			0.13	0.14	0.34	0.11	9.07	1.86	15.78
Standard Dev.=			8.77	9.55	0.23	7.76	6.23	1.28	10.79
CV =			6.98	2.81	5.38	1.59	53.22	6.15	11.57

[0]Julian application dates were 192 (10 Jul) and 207 (25 Jul) for 2 applications and 192 (10 Jul), 207 (25 Jul), 242 (29 Aug), and 252 (8 Sep) for 4 applications.

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Julian dates for GLS DSI scored during the growing season.

[3]Yield expressed as kg/ha at a standard 15.5% moisture content.

[4]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 5. Fungicides for control of gray leaf spot (GLS) of corn in 1990.

Fungicide	Rate	no. of[0]	Disease Severity Index (DSI) (0-5)[1]				%	%	Yield
	g ai/ha	applic	229[2]	243	261	271	lodged	H_2O	kg/ha[3]
Nontreated		0	2.22a[4]	3.57a	4.77a	4.96a	1.5ab	16.3c	5434.6b
Propiconazole	126	2	1.58bc	2.38b	3.88b	4.83a	3.0a	18.1ab	6601.9de
Propiconazole	126	4	1.65b	2.05bcd	3.20cd	3.23de	2.4ab	19.2ab	7681.3a
Terbutrazole	126	2	1.50bc	2.13bc	3.85b	4.55b	0.9ab	17.5bc	7034.9a
Terbutrazole	126	4	1.42bcd	1.90cde	2.90de	3.22de	0.0b	18.1ab	6972.1a
RH-7592 2.0F	126	2	1.52bc	1.90cde	3.13cde	3.34cd	1.3ab	19.4a	7298.5a
RH-7592 2.0F	126	4	1.63bc	1.82cde	2.72e	3.03e	2.6ab	19.1ab	7216.9a
DPX-H6573 25EC	126	2	1.20de	1.85cde	3.23cd	3.58c	1.9ab	18.4ab	6921.9a
DPX-H6573 25EC	126	4	1.38cd	1.73cde	2.42f	2.63f	1.4ab	18.8ab	7292.2a
Benomyl	560	2	1.05e	1.67de	3.35c	3.48cd	1.7ab	19.3ab	7417.7a
Benomyl	560	4	1.00e	1.58e	2.45f	2.58f	1.2ab	19.1ab	7618.5a
LSD (0.05) =			0.24	0.37	0.41	0.27	2.38	1.56	18.07
Standard Dev.=			0.17	0.25	0.28	0.19	1.65	1.08	12.52
CV =			11.3	12.42	8.64	5.26	102.4	5.84	11.12

[0]Julian application dates were 201 (19 Jul) and 229 (16 Aug) for 2 applications and 201 (19 Jul), 229 (16 Aug), 244 (30 Aug), and 261 (17 Sep) for 4 applications.

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Julian dates for GLS DSI scored during the growing season.

[3]Yield expressed as kg/ha at a standard 15.5% moisture content.

[4]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 6. Fungicides for control of gray leaf spot (GLS) of corn in 1991.

Fungicide	Rate	no. of[0]	Disease Severity Index (DSI) (0-5)[1]				%	%	Yield
	g ai/ha	applic	207[2]	225	247	264	lodged	H_2O	kg/ha[3]
Nontreated		0	1.6a[4]	2.7a	4.8a	5.0a	2.8a	15.2f	3175.4g
Propiconazole	126	2	1.1abc	1.9b	3.5bc	4.9a	0.9a	16.1ef	4531.0f
Propiconazole	126	4	1.1abc	0.8c	2.3d	3.3cd	1.8a	17.8cd	5428.4def
Terbutrazole	126	2	0.9c	1.9b	3.7b	4.5ab	0.8a	16.8de	4731.8ef
Terbutrazole	126	4	1.4ab	0.9c	2.1d	3.5c	0.0a	18.2bc	5566.4def
RH-7592 2.0F	126	2	1.0bc	1.5bc	2.2d	2.6de	1.6a	19.3ab	6012.0a-d
RH-7592 2.0F	126	4	1.4abc	0.8c	0.9ef	1.5fg	3.0a	19.7a	6991.0ab
DPX-H6573 25EC	126	2	1.1abc	1.3bc	3.1e	4.0bc	2.4a	17.9cd	5792.3cde
DPX-H6573 25EC	126	4	0.9c	0.7c	1.2e	2.1ef	1.5a	19.1ab	5786.1b-e
Benomyl	560	2	0.9c	0.9c	2.1d	2.5de	2.0a	18.8abc	6840.4abc
Benomyl	560	4	0.9c	0.7c	0.7f	1.3g	1.4a	19.8a	7085.1a
LSD (0.05) =			0.46	0.81	0.51	0.75	3.7	1.0	17.1
Standard Dev.=			0.32	0.56	0.35	0.51	2.57	0.70	11.8
CV =			27.87	44.01	14.82	16.21	156	3.83	13.16

[0]Julian application dates were 191 (9 Jul) and 207 (25 Jul) for 2 applications and 191 (9 Jul), 207 (25 Jul), 225 (12 Aug), and 247 (3 Sep) for 4 applications.

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Julian dates for GLS DSI scored during the growing season.

[3]Yield expressed as kg/ha at a standard 15.5% moisture content.

[4]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

To determine sources of DSI interaction between years, fungicide treatments, common to each year were statistically analysed by GLM. Common fungicide treatments are listed in Table 7. The ANOVA of gray leaf spot DSI indicated that ratings differed significantly with a $P \leq 0.0001$, because of year, fungicide, and rating date. A significant interaction between year and rating date showed that environment may have affected the epidemiology of GLS. The F value of 21.95 was highly significant at the 0.0001 level (Table 2).

Table 7. Fungicide treatments, common to 1989, 90, and 91, effect on gray leaf spot (GLS) DSI and grain yield (kg/ha).

Fungicides	Rate	no. of[0]	Disease Severity Index (DSI) (0-5)[1]				Yield[2]
	g ai/ha	applic	207-229[3]	225-243	247-261	264-271	kg/ha
Nontreated		0	1.86a[4]	3.43a	4.72a	4.94a	4406.1d
Propiconazole	126	2	1.25cd	2.48b	3.54b	4.62a	5770.4c
Propiconazole	126	4	1.30c	2.02cd	2.92c	3.45b	6453.8bc
Terbutrazole	126	2	1.18cd	2.48b	3.70b	4.48a	6147.4c
Terbutrazole	126	4	1.50b	2.38bc	2.93c	3.22ab	5624.2bc
Benomyl	560	2	1.04d	1.96cd	2.91c	3.39b	6798.9ab
Benomyl	560	4	1.03d	1.79d	2.08d	2.44c	7310.4a
LSD (0.05) =			0.20	0.41	0.41	0.75	10.72
Standard Dev.=			0.24	0.50	0.50	0.92	13.13
CV=			18.69	21.15	15.48	24.82	13.48

[0]Application dates were pooled over all three years; for individual application dates see Tables 1-3.
[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.
[2]Yield expressed as kg/ha at a standard 15.5% moisture content.
[3]Four GLS DSI rating periods bracketed by julian dates, *ie.*, 207-229 (Jul to mid-Aug), 225-243 (mid-late Aug), 247-261 (early-mid Sep), and 264-271 (late Sep).
[4]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

The progress of GLS severity for the nontreated check and the benomyl fungicide treatment with four applications during 1989, 90, and 91 growing seasons illustrated the development of blighting over time for the nontreated check and the most efficacious fungicide treatment (Figure 1). By comparing the nontreated check responses to environment and GLS severity, explanations for the significant year by rating date interaction were assessed. After planting in 1989, ample periods of rain (12 cm) (Figure D.2) and moderate temperatures averaging 25 C (Anonymous, 1989) were sufficient for kernel set. By 25 July, GLS lesions had progressed passed the ear leaf and continual episodes of rainfall (≥ 10 cm), lasting as long as 18 days with moderate temperatures (Anonymous, 1989) continued for the remainder of the growing season. Prolonged periods of overcast skies were also prevalent during the growing season. These conditions were highly conducive to GLS development as shown in Figure 1. Severity surpassed that of years 1990 and 91. Overcast skies and blighting may have interfered with the corn plants ability to capture light energy and produce sugars, thus negatively affecting ear development (Aldrich, et al., 1986). This could have accounted for significant yield decreases in spite of the hurricane that had occurred in September of 1989.

In May 1990 the 18.2 cm (Anonymous, 1990) of rain recieved after planting was sufficient for early vegetative growth. However, the month of June was extremely dry with intermitant rainfall totalling approximately 4 cm (Anonymous, 1990). This may have attributed to the retardation of GLS disease development (Figure 1). Plots in July

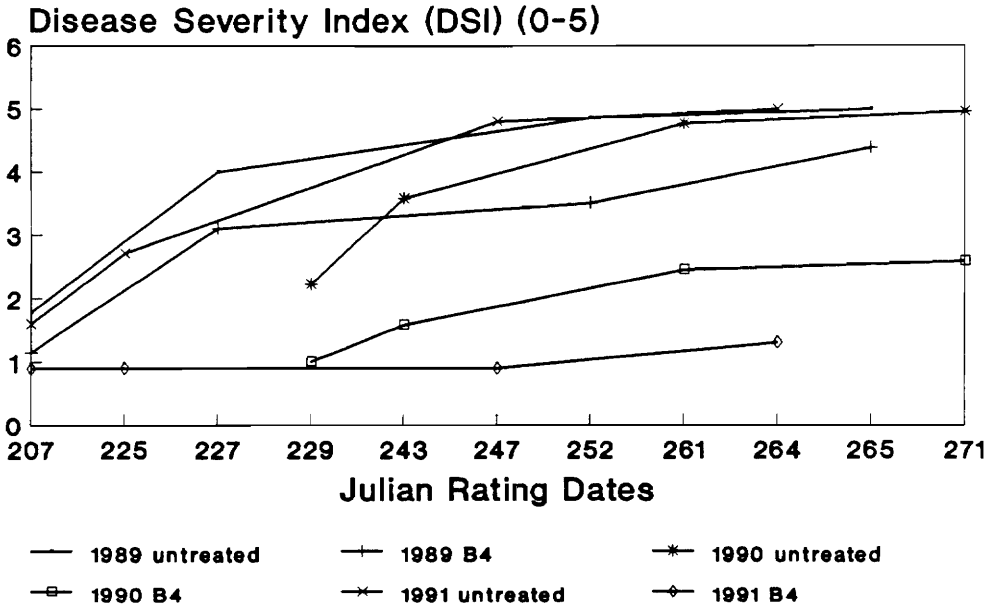

Figure 1. DSI of nontreated susceptible hybrid and benomyl fungcide (B4) applied 4 times from Julian dates 207-271 in 1989, 1990, and 1991.

received 9.4 cm of rain over three days accompanied by hot temperatures ≤ 33.3 C (Anonymous, 1990). Rainfall of 5.8 cm within two days prevailed in early August along with averaged weekly temperatures of approximately 25 C (Figures B.1 and D.2). All other days during this period were characterized as being unseasonably dry and hot. Drouthy conditions may have interferred with sporulation of *C. zeae-maydis* dramatically throughout the growing season. Similarly, Rupe *et al.* (1982) showed that short periods of dryness resulted in death of *C. zeae-maydis* conidia on glass slides.In late-August to early-September, rainfall was plentiful (Figure D.2) and conditions were conducive to GLS development. Thus a rapid increase in blighting resulted in September (Figure 1).

In 1991, rainfall in May was plentiful, as in preceding years (Figure D.2), with moderate averaged weekly temperatures of 24.5 C inducing adequate conditions favorable for vigorous vegetative growth. During the month of June to 11 July, conditions were extremely dry with only 2.4 cm of rain, possible retarding GLS development (Figure 1) and interferring with ear and tassel formation. By mid-August to early-September rainfall decreased to three short rainy periods, each not exceeding 0.94 cm (Anonymous, 1991). Averaged weekly temperatures ranged from 25 C to 33.3 C in periods of as many as 18 days without rain (Figures B.1 & D.2). These weather conditions are likely to interfere with pollination and transpiration which could result in poor kernel set. These observations attribute variation between rating date and year to the environments effect on GLS epidemiology and hybrid vigor.

The combined ANOVA of grain yield from evaluations of the common fungicide

treatments and water control for 1989, 90, and 91 shows that year, fungicide and the number of applications were highly significant ($P \leq 0.0001$). This would be expected from the varying levels of disease control and enviromental effects (Table 3).

Nevertheless, severity of blighting ranged from 0.7-4.82, as measured by DSI, on treated plants to 4.77-4.86 on nontreated plants by the early to mid-September rating. Benomyl with two and four applications was significantly the most effective. Yields from plots treated with benomyl were almost twice that of the check, however, all high yield and low DSI potential of benomyl treatments may not be attributed to controlling GLS disease alone. Benomyl has weak cytokinin-like properties that may have delayed senescence (Schruft, 1971; Skene, 1972; Thomas 1873, 1974), thus may have increased the amount of photosynthate available for grain fill. Propiconazole and terbutrazole with two applications increased yields by 30-34% over the nontreated control. Significant yield increases and blight reductions resulted whether applications were applied two or four times during the growing season. One or two applications may result in adequate control of GLS disease for use in seed corn production fields.

Mean yields for the six common fungicide treatments and the nontreated water control combined over all years were regressed against the DSI at the July to mid-August rating period (Figure 2). An 87% correlation of blighting to grain yield showed a slope of -3049.48. For the mid-late August rating period (Figure 3), a 93% correlation of grain yield loss was attributed to blighting. These mid-season DSI's have been the most reliable predictor of the effect of blight on grain yield. This agrees with observations of

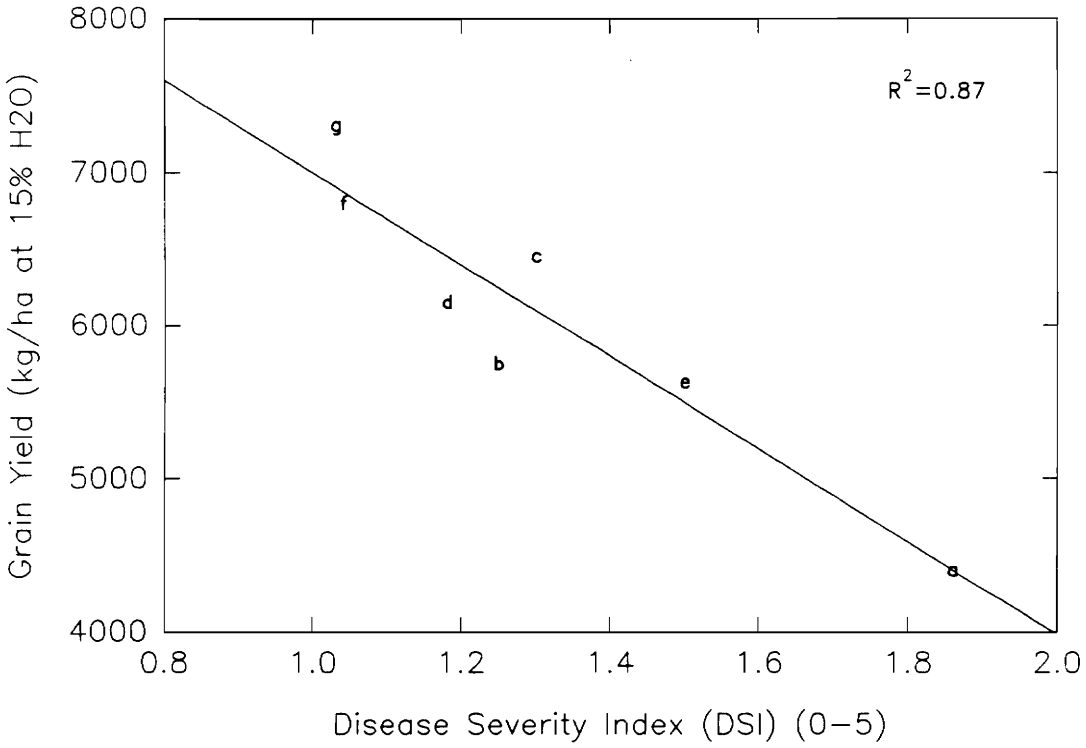

Figure 2. Comparison of grain yield and DSI in July mid–August (Julian dates: 207–229) for common fungicide treatments over three years; where a = nontreated check, b = terbutrazole applied 4 times, c = propiconazole applied twice, d = terbutrazole applied twice, e = propriconazole applied 4 times, f = benomyl applied twice, and g = benomyl applied 4 times.

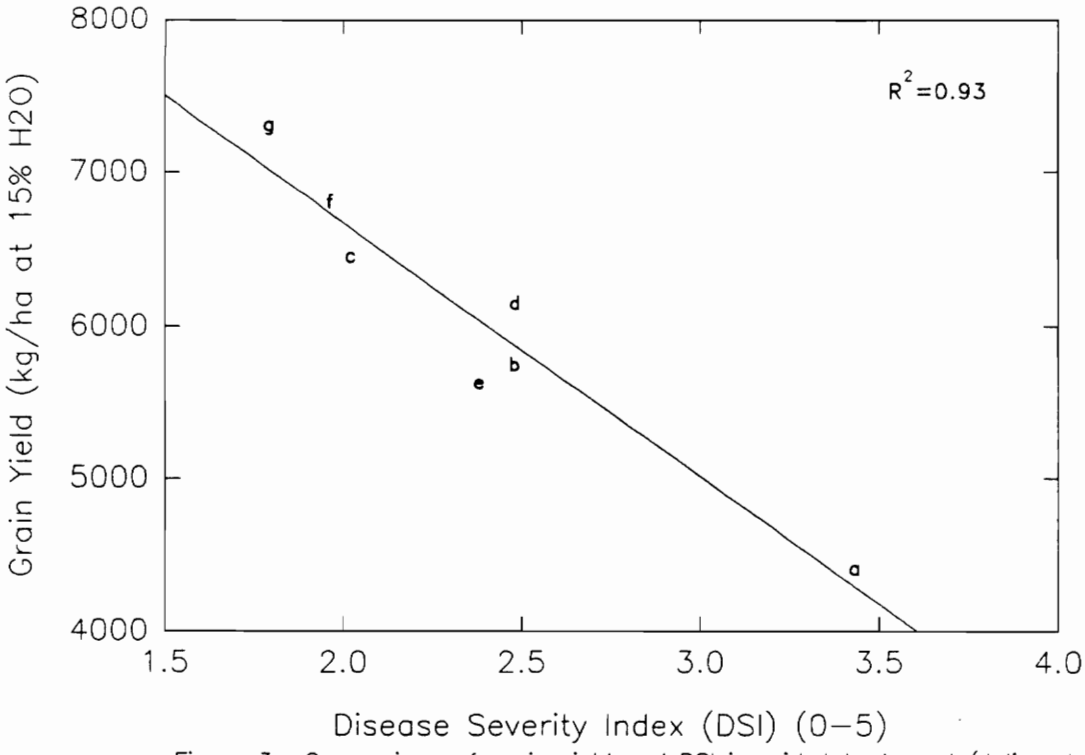

Figure 3. Comparison of grain yield and DSI in mid–late August (Julian dates: 225–243) for common fungicide treatments over three years; where a = nontreated check, b = propiconazole applied twice, c = propiconazole applied 4 times, d = terbutrazole applied twice, e = terbutrazole applied 4 times, f = benomyl applied twice, and g = benomyl applied 4 times.

Stromberg and Donahue (1986) for hybrid resistance and yield. By comparing yield with DSI from the early to mid-September ratings (Figure 4), showed a lower correlation for blighting to yield (R^2 = 0.80) occurred and an R^2 value of 0.46 by late-September resulted (Figure 5). These decreases for the effect of blight on yield with the late season ratings may be due, in part, to senescence. Senescence may interfere with visual ratings for disease.

Five-hundred kernel weights were obtained in 1990 and 91. Highest kernel weights were obtained from RH-7592 (189.8-196.8g) and DPX-H6573 (187.5-194.0g), and benomyl (153.8-154.5g) in 1990 and 1991, respectively. The number of applications did not significantly ($P \leq 0.05$) affect kernel weights when compared with individual fungicides in each year. A significant ($P \leq 0.05$) increase among kernel weights were obtained from treated verses nontreated corn plants and resulted in 7 to 26% increases in 1990 and 91, respectively. However, kernel weights were significantly higher ($P \leq 0.0001$) in 1990 than 1991. This may be, in part, attributed to 1991's dry climate that may have limited yield potential. Thus, grain weights were regressed separately by year for assessment of kernel weight loss due to GLS severity. In 1990 the mid-late August rating resulted in a negative correlation between kernel weight and disease severity with an R^2 of 0.76. This correlation decreased by the late-August to early-September rating period to an R^2 of 0.69. In 1991, 81% of the yield loss could be attributed to GLS blighting in mid-late August and 75% in late-August to early September. Thus, grain yields for each year were regressed separately against kernel weight for determination of

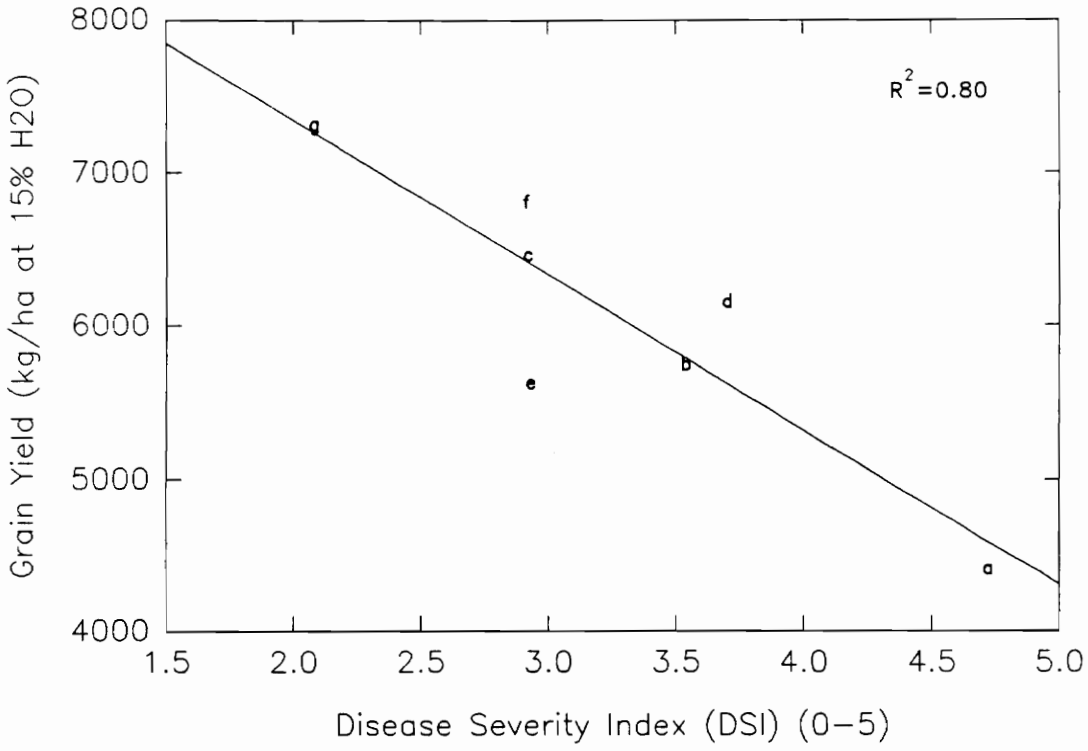

Figure 4. Comparison of grain yield and DSI in early-mid September (Julian dates: 247-261) for common fungicide treatments over three years; where a = nontreated check, b = propiconazole applied twice, c = propiconazole applied 4 times, d = terbutrazole applied twice, e = terbutrazole applied 4 times, f = benomyl applied twice, and g = benomyl applied times.

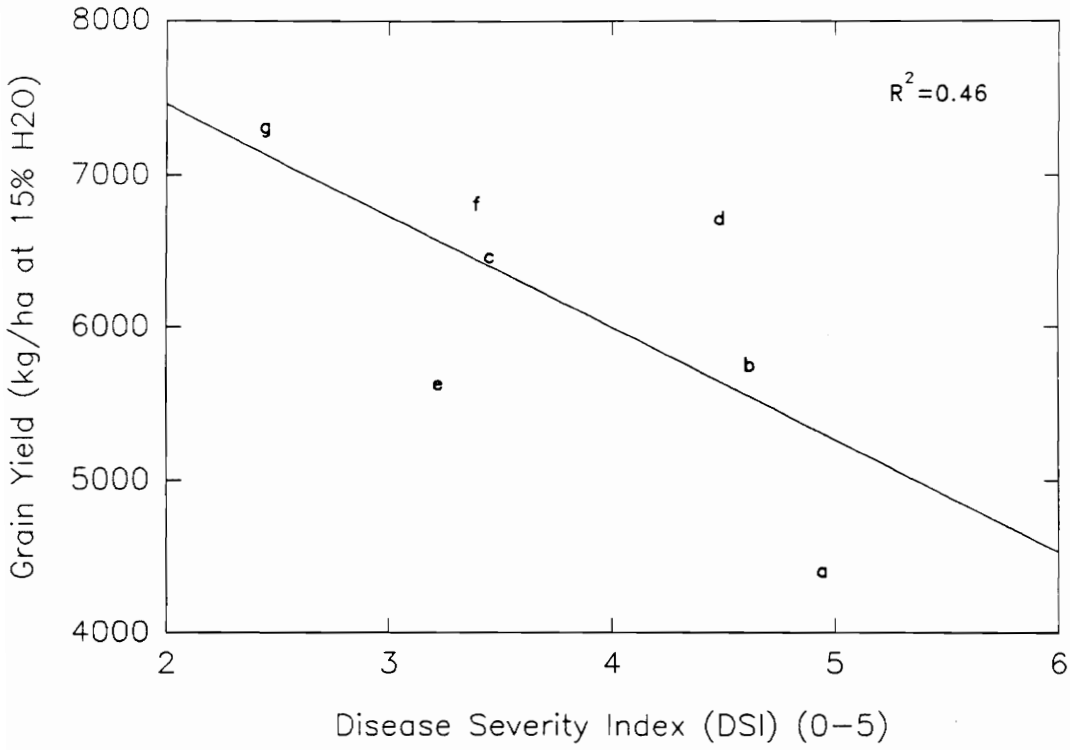

Figure 5. Comparison of grain yield and DSI in late–September (Julian dates: 264–271) for common fungicide treatments over three years; where a = nontreated check, b = propiconazole applied twice, c = propiconazole applied 4 times, d = terbutrazole applied twice, e = terbutrazole applied 4 times, f = benomyl applied twice, g = benomyl applied 4 times.

kernel weight effect on total grain yield (Figure 6 A and B). Only 57% of the grain yield loss could be attributed to kernel weight in 1990 (Figure 6 A). However, 92% of the grain yield loss was due to kernel weight in 1991 (Figure 6 B). These evaluations resulted in a positive correlation between kernel weight and grain yield.

Moisture content of seed at harvest significantly differed between 1989, 1990, and 1991 at $P \leq 0.0001$. Results indicated that grain moisture was positively correlated to grain yield in kg/ha with R^2 values of 0.29 (1989), 0.87 (1990) and 0.88 (1991). This indicates that fungicide treatments decreased blighting, thus permitting more photosynthate to be directed to ear fill than the nontreated check. This indicated an extended grain filling period with the use of fungicides for the control of blighting. These results confirm those of Donahue and Stromberg (1986) who determined that more resistant hybrids have less blighting, higher kernel moisture and higher grain yields adjusted to a standard moisture content. Many plants lodged in 1989 as a result of hurricane Hugo. Thus, a direct correlation between percentage moisture and grain yield of 1989 was confounded.

Percentage leaf area blighted by GLS disease, as measured on the ear leaf, was determined on 24 September, 1991 (Julian date 268). Regression of grain yield and percentage ear leaf area blighted indicated that 82% of grain yield loss could be attributed to percentage leaf area affected (Figure 7), a substaintally higher correlation than that of the September DSI rating in 1991 (Figure 5). This suggests that since GLS disease severity was low, percentage ear leaf area blighted may to be a better method for

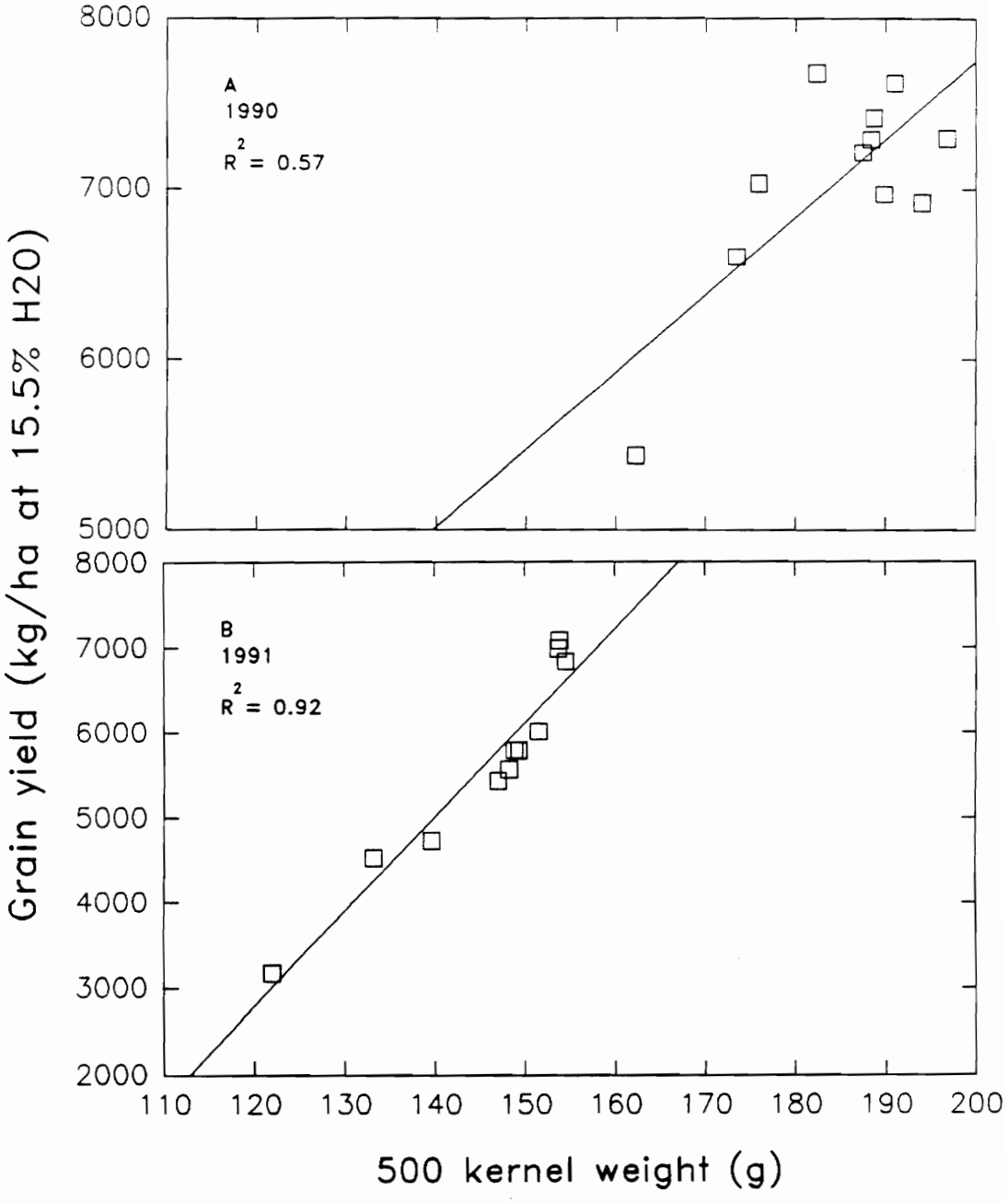

Figure 8. comparison of grain yield and 500 kernel weight for fungicide treatements used in 1990 (A) and 1991 (B).

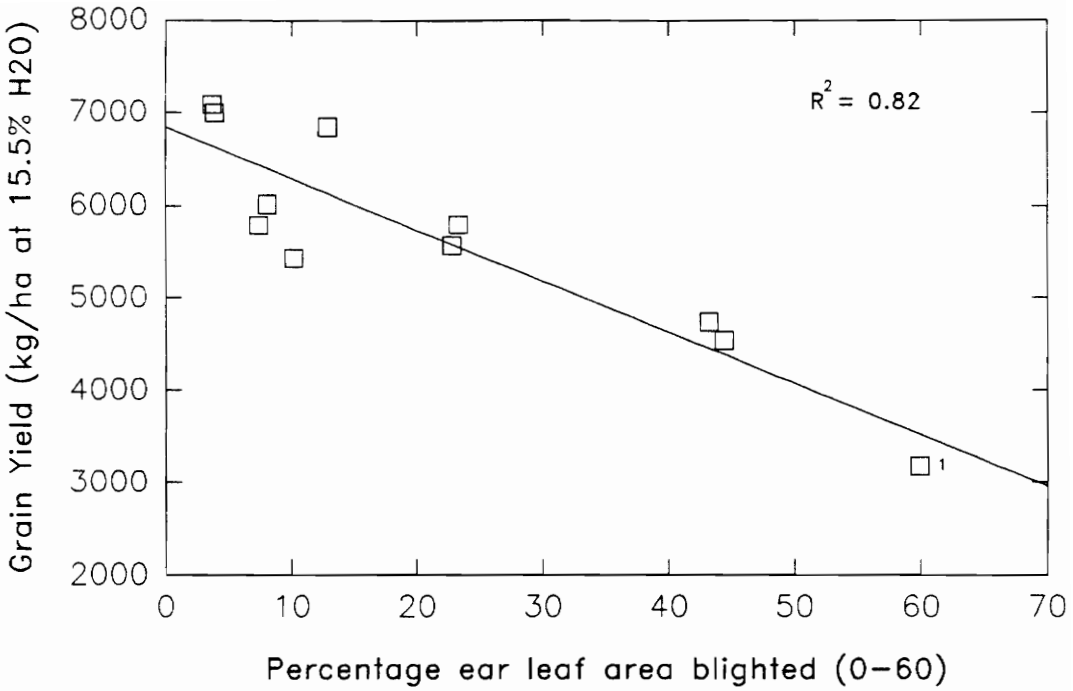

Figure 7. Comparison of grain yield and percentage ear leaf blighted for fungicide treatments used in 1991 (1 = nontreated check).

determination of the GLS disease severity late-season effect on yield than that of DSI.

In conclusion, the use of systemic foliar fungicides, *ie.*, benzimidazole and triazoles, reduce blighting caused by GLS disease of corn. This reduction in blighting increases grain moisture, kernel weight and grain yield. Yield loss caused by GLS disease is affected by environmental conditions throughout the growing season. These losses in seed corn production may be severe, particularly if the female parent is susceptible to GLS. This study indicates that even under conditions less than conducive to GLS, use of systemic foliarly applied fungicides may significantly increase yield and kernel weight.

Alternatively, the use of more resistant hybrids also reduces blighting and keeps the corn plant green during the important grain producing periods (Stromberg and Donahue, 1986). Hybrids with varying levels of resistance have been evaluated for their performance under high levels of GLS disease pressure and significant increases in yield resulted from hybrids with less blighting as shown in Chapter 1 of this thesis.

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Germplasm Sensitivity to Cercosporin and Implications for Gray Leaf Spot Resistance

by

M.R. Carter

ABSTRACT

Cercospora zeae-maydis (CZM), causal agent of gray leaf spot (GLS) of corn, produces cercosporin, a red-colored, light-activated toxin, that may play a role in GLS development. Germplasm varying in degree of resistance to GLS were evaluated for disease severity induced by CZM in the field. Responses were compared to results obtained from three methods used to elicit germplasm sensitivity to cercosporin, *ie.*, field and glasshouse vein inoculation, and root and shoot uptake. Vein inoculation studies did not result in consistant differential responses between hybrids, but did indicate that 39-day old plants were significantly ($P \leq 0.0001$) more sensitive than 21-day old plants, as measured by lesion width. Root and shoot uptake of toxin produced water soaked leaf tips by 38h and became necrotic with chlorotic borders after 72 h. Lesions resembled those produced by CZM in the field. Under fluorescence microscopy, yellow fluorescing crystals were observed within necrotic leaf tissue of treated plants. In some leaf lesion extracts, 407.1-1076.7 ng cercosporin/g fresh weight of tissue was recovered by chromatographic analysis. Shoot uptake of toxin produced variable responses between germplasm. Only five inbreds (B73, H99, Va59, NC264, and NC250a) showed consistant and differential responses, and interestingly, cercosporin produced greater injury in NC250a than in H99 with similar amounts of toxin recovered. This suggests that some germplasm may be more sensitive to cercosporin than others. Unfortunately, germplasm sensitivity to the toxin using these techniques were not indicative of germplasm response to CZM in the field.

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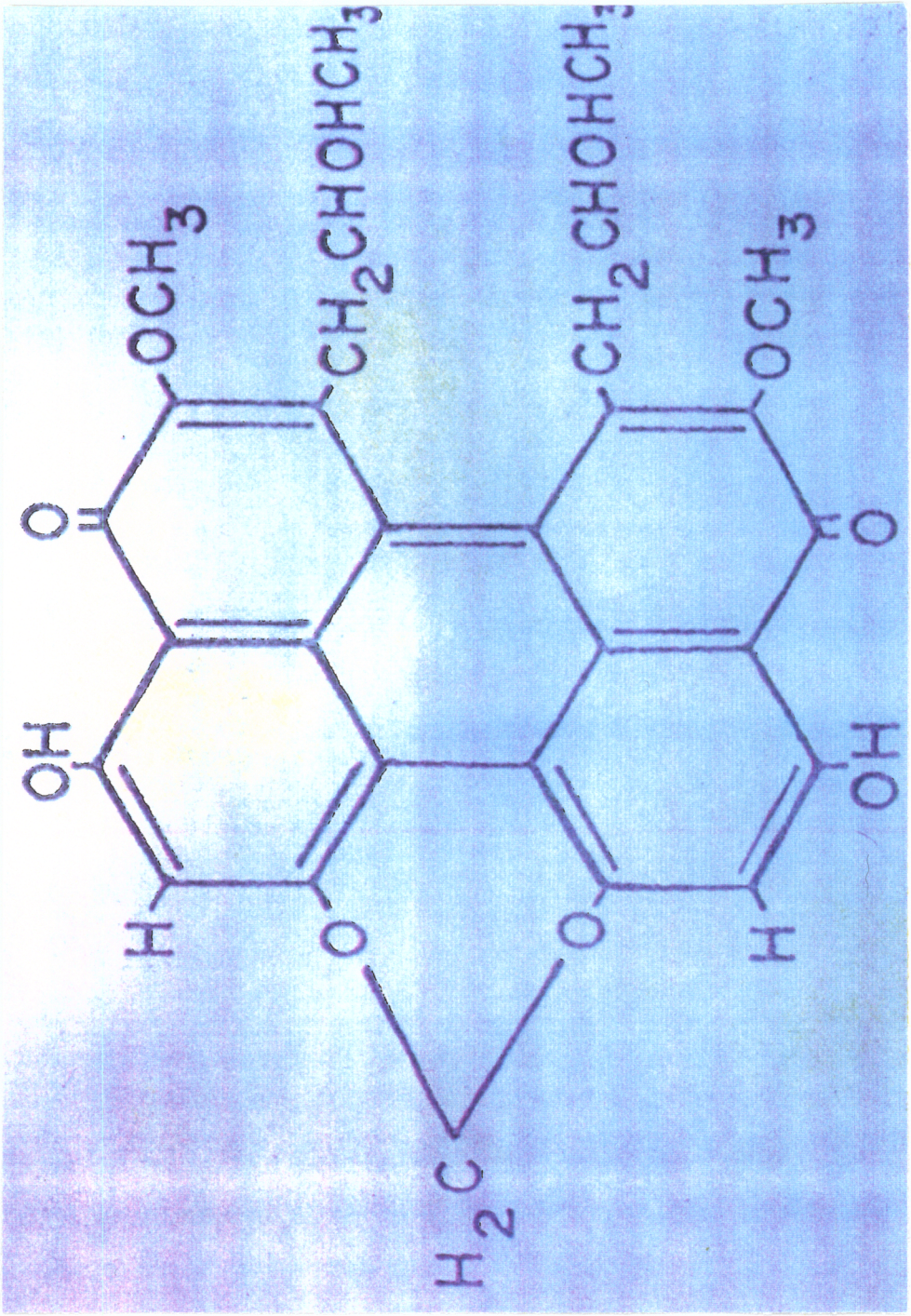
Gray leaf spot (GLS) disease of corn caused by the fungus, *Cercospora zeae-maydis* (Tehon & Daniels, 1925), characteristically produces necrotic rectangular foliar lesions surrounded by a yellow halo, early in its develoment. As these lesions mature they become vein-bound with a gray cast due to sporulation of the pathogen on the necrotic tissue.

Members of the genus *Cercospora* produce a toxin, cercosporin, which is a red, light activated phytotoxic chemical (Assante *et al.*, 1977; Balis and Payne, 1971; Fajola, 1978; Lu, 1983, Lynch and Geoghegan, 1977, Mumma *et al.*, 1973) (Color plate 1). In the presence of light cercosporin is converted to a triplet state that induces superoxide ions and singlet oxygen (Daub, 1987). These species are toxic to living cells, causing oxidation of cellular constituents, including proteins, carbohydrates and nucleic acids (Daub, 1987; Spikes, 1977).

Two factors appear to link cercosporin to development of disease caused by *Cercospora* species. One is that light is required for symptom development in *Cercospora* incited diseases (Calpouzos, *et al.*, 1963; Calpouzos and Stallknect, 1967; Steinkamp, *et al.*, 1981). Second, cercosporin, by itself, produces necrotic lesions similar to those observed with *C. beticola* on sugar beet (Steinkamp, *et al.*, 1979, 1981).

In other *Cercospora* spp., this toxin has been shown to be a key pathogenicity factor (Upchurch, *et al.*, 1989). *Cercospora* spp. grow intercellularly within host tissue. They are facultative saprophytes that apparently use cercosporin to kill the host tissue in advance of the fungus, thus increasing their ability to colonize leaf tissue.

Color plate 1. Structure of cercosporin (Daub, 1982).


OCH3
CH2CHOHCH3
O
OH
H
O
H2C
O
CH2CHOHCH3
OCH3
O
OH
H

Application of purified toxin has been shown to produce disease-like symptoms in susceptible hosts. Gwinn *et al.* (1987) emersed leaf disks from three corn genotypes into a 1.2μM cercosporin solution and concluded that no varietal differences were detected. Procedures for screening germplasm for disease resistance are generally performed in the field on mature plants infested with *C. zeae-maydis*. This method does permit analysis of disease expression, but does not indicate sensitivity of germplasm to cercosporin. Field observation of various lesion sizes among different germplasm suggests that lesion size is restricted in some germplasm and this may involve plant induced resistant responses to cercosporin.

Mechanisms of resistance to gray leaf spot may involve the neutralization of or host tolerance to the fungal toxin. All plants contain compounds that serve as free radical quenchers, *eg.*, carotenoids, anthocyanins, and superoxide dismutase, which are produced by plants under stress or during photosynthesis. Stromberg and Donahue (unpublished) have correlated reduced lesion size with a halo of red pigment bordering lesions of more resistant plants. These pigments could include carotenoids, whose role as a free radical quencher has been demonstrated by Krinksy (1979), or anthocyanins. The similarity in absorption optimum for anthocyanins and cercosporin suggest that anthocyanin could function to absorb photoactivating wavelengths, (Chappel and Hahlbrock, 1984) and reduce the toxic activity of cercosporin. Superoxide dismutase has been shown to reduce stress from free radical production and oxidative stress (Rabinowitch and Fridovich, 1983; Matters and Scandalios, 1986). Daub (1987) reported that tobacco cell lines

selected for resistance to the free radical producing herbicide, paraquat, and high levels of SOD, showed increased tolerance to cercosporin. Mutants (Miller and Hughes, 1980) selected from tobacco cell culture for resistance to paraquat showed decreased sensitivity to cercosporin (Daub, unpublished). All germplasm previously tested has shown sensitivity to cercosporin. Incorporation of cercosporin resistance into corn germplasm could provide a novel source of resistance to *Cercospora*. Therefore, the role of cercosporin in resistant germplasm should be tested. This study compares germplasm resistance and susceptibility to gray leaf spot in the field with germplasm sensitivity to cercosporin performed in the greenhouse and other screening procedures.

MATERIAL AND METHODS

Genetic Material

Inbreds varying in resistance and susceptiblity to GLS from field evaluation, were obtained as follows; Pa875 and Pa91 (Pennsylvania State Univerisity, Univerisity Park, PA), NC250, NC250a, NC264, NC268, (North Carolina State University, Raleigh, NC) B68, B73, H99, A632, Mo17, H93 (Wilson Hybrids, Harlan, IA) and Va22, Va59, and Va14 (Virginia Polytechnical Institute and State University (VPI&SU), Blacksburg, VA) for GLS response assessment. Hybrids from Donahue (1986), *ie.*, B73 X Pa91 and B68 X Va14 and commercial hybrids (Table 2) from various seed companies were used for preliminary cercosporin evaluations.

Inbred field trial

Field plot. Inbreds were planted in a randomized complete block (RCB) with three

replications in Montgomery County, Virginia on 24 May, 1991. The plot design was arranged in two-row plots, 4.57m in length spaced 0.76m apart. Two-row plots consisted of one row of susceptible hybrid, Pioneer Brand 3320, and one row of a randomly selected inbred. *Cercospora zeae-maydis* infested corn debris obtained from the Whitethorne-Kentland Plantation Experimental Farm in Montgomery County, Virginia was introduced to the field site in 1987. Inoculum levels had been maintained at high levels by continuous no-tillage practices in previous years with a susceptible hybrid, Pioneer Brand 3320. Prior to planting, a complete fertilizer (47-23-30 NPK at 493.2 kg/ha) was applied based on soil analysis. During the 1991 growing season, approximately 10 hours of irrigation/day were administered from July-August in response to drought.

Data collection. Inbreds were evaluated for differential responses to GLS using a Disease Severity Index (DSI) from 0-5 described by Roane *et al.* (1974) and modified by Hilty *et al.* (1979). For this index, 0 = no symptoms, 1 = trace of lesions below the ear but no lesions above the ear, 2 = many lesions below the ear but only a trace of lesions above the ear, 3 = severe lesion development below the ear and all the leaves above the ear have some lesions, 4 = all the leaves show severe lesion development but green tissue is still visible, and 5 = all leaves are dry and dead. Assessments of blighting was obtained four times during the growing season on 2 August, 16 August, 3 September, and 20 September, 1991.

Cercosporin purification

Cercosporin was obtained by culturing *Cercospora zeae-maydis* mycelia, on liquid Whites medium (White, 1963). The following cercosporin extraction procedure was modified from Jenns (1989). Freeze-dried mycelia were pulverized and extracted with 5N KOH. The mycelial extract was acidified with equal volume of 5N HCL, filtered through Whatman No.1 filter and filtrate collected. Equal volumes of chloroform were added to the filtrate, the organic phase was collected, and evaporated to dryness on a rotary evaporator. The cercosporin sample was redissolved in ethanol. Purified cercosporin was provided by Dr. David Radin and his technician, Wendy Baur, at VPI&SU, Blacksburg, VA.

To determine the concentration of cercosporin diluted in an ethanol or acetone carrier, spectrophotometry was used at two wavelengths, 473 and 565 nm on a Beckman DU65 Spectrophotometer (Beckman Instruments, Inc.).

Preliminary vein inoculation with cercosporin in the field

Elite hybrids (Table 2) were planted on 23 May, 1991 at final population of 52800 seeds/ha at Whitethorne Kentland Plantation Expermental Farm, Blacksburg, Virginia. The plot consisted of four rows 7.6m in length, with rows spaced 0.76m apart arranged in a randomized complete block (RBC) design with four replicates. Five randomly selected plants from each replicate were treated with 2.5mM of cercosporin solution dissolved in 50% ethanol (v\v) and a 50% ethanol:water (v/v) control. Tertiary veins on either side of the midvein were severed with a 20 guage syringe tip immediately

preceding an application of either 1 drop from the cercosporin or ethanol treatment. A lower leaf (sixth leaf from the bottom) received an application 50 days after planting (DAP) and an upper leaf (third leaf from the top) received treatments 77DAP. Lesions were measured according to the following parameters; total length, width at the widest point, length from point of inoculation toward stalk, and length from point of inoculation toward leaf tip in millimeters and recorded at 5 and 14 days after treatment (DAT) for both sets of leaves.

Glasshouse experiments

Seedling propagation. Twenty-four seeds were placed on moist paper towels in 15 X 2.5 cm petri plates in the darkness for 5 days. Germinated seeds were transferred to 10 cm pots containing Spasoff mix (37.8 l Weblite, 18.9 l Vermiculite, 9.5 l peat moss, 454 g osmocote, 227 g lime, and 227 g NPK 10-10-10) planted approximately 3.8 cm deep. Nutrients were supplied by adding 25 ml of a modified Hoagland's solution (Hoagland and Arnon, 1938) to each seedling. Glasshouse temperature ranged from 23.9-32 C.

Vein inoculation of cercosporin in the glasshouse. Four hybrids, Wilson Demand 110, Pioneer Brand 3192, Pioneer Brand 3154, and Pioneer Brand 3320 were selected by their known field response to GLS disease (Stromberg, 1990a, 1990b; Stromberg and Carter, 1991a, 1991b; and Stromberg and Donahue, 1986) and cercosporin induced lesion response as indicated by the preliminary vein inoculation trial in the field. Three tertiary veins on one side of the midrib were slit with a 20 guage syringe needle and 1 µl of 1.2mM cercosporin solution dissolved in 50% ethanol (v/v) or a 50% ethanol:water (v/v)

alone were applied with a 10µl Hamilton syringe on the third and fifth leaf of 21- and 39-day old seedlings, respectively. Lesion measurements were similar to those of the field vein inoculation study previously described and were determined at 1, 3, 6, and 8 DAT.

Growth chamber experiments. A I-60DL Dew Chamber (Percival Manufacturing Co., Boone, Iowa) with a Cam-operated Recording Temperature and Humidity Programmer, Model RFC 52 (Partlow Corporation, New Hartford, New York) was used for all growth chamber experiments. The dew chamber was programed at a constant 25 ± 2C with low relative humidity (approximately 15%) for 12 hour alternating night/day cycles. Light intensity measured 92.9 lux.

Assessment of injury was determined for corn seedlings exposed to various concentrations of cercosporin or the ethanol or acetone control solutions at the second to fourth leaf stage. Ten categories of injury expression are decribed as: 0 = no visible injury; 1 = slight chlorosis on the first vegetative leaf; 2 = chlorosis and slight necrosis on the first vegetative leaf; 3 = first vegetative leaf shows chlorosis with severe necrosis and wilting on second, third, or fourth vegetative leaf; 4 = first vegetative leaf necrotic and other vegetative leaves appear wilted; 5 = watersoaked appearence or plant injury just beyond leaf collars; 6 = watersoaked patches appearing on leaf tissue, usually at or close to leaf tips, approximately 60% of the seedling injured; 7 = general chlorosis or watersoaked appearence on second, third, or fourth vegetative leaf, approximately 70% of the seedling injured; 8 = watersoaked or chlorotic areas turn brown at tips or in the center of tissue injury on some or all leaves, approximately 80% of the seedling injured;

9 = entire seedling chlorotic and/or necrotic.

Root-uptake of cercosporin. The root-uptake procedure was modified from Harvey, *et al.* (1978), Blaney, *et al.* (1988) and Shaner, *et al.*, (1985). Hybrids were chosen for preliminary testing of a more susceptible (B73 X Pa91) and a more resistant (B68 X Va14) hybrid to GLS for their level of sensitivity to cercosporin. These hybrids were also used to determine the value of the root-uptake method in evaluating germplasm for sensitivity to cercosporin. At 5 days old, seedlings were gently uprooted and their roots were directly submersed into aluminum foil covered test tubes containing glass beads and 5 ml of Hoagland's solution. When the plants reached 11 days old, the Hoagland's solution was removed and replaced by 5, 10, and 50 μM of a cercosporin solution dissolved in 1, 5, or 25% ethanol (v/v), respectively, or a 25% ethanol:water (v/v) control. In a second experiment, root tips were excised with a razor blade under water, immediately prior to submersion of roots into the same solutions as above. Visual observation of injury were noted daily at 4 or 8 hour intervals over a 72 hour period, for both experiments.

Pioneer Brand 3320 alone was subjected to cercosporin treatment by pruning roots and transferring seedlings to 10, 50, or 100μM cercosporin concentrations dissolved in 5, 25, and 50% ethanol (v/v), respectively, with 50% ethanol:water (v/v) as a control. A replicate experiment was placed in a dark cabinet at room temperature (22 ± 2C). Experiments were observed periodically for injury during a 96 hour period.

All root uptake samples were harvested immediately after injury data were

recorded by sectioning leaf, shoot and root tissue and combining replications for which fresh weights were recorded. Samples were stored at -80 C for later pigment extraction. Small leaf, shoot, and root pieces were also air-dried or observed directly after harvest by light and fluorescence microscopy for presence of cercosporin.

Shoot-uptake of Cercosporin. Three preliminary experiments were conducted to evaluate the shoot uptake procedure. Shoots from Pioneer Brand 3320 and B73 seedlings, at 14 days of age, were excized under water and transferred directly into glass tubes containing 15, 25, 35, or 45μM cercosporin concentrations dissolved in 1.6, 2.7, 3.8, or 4.8% ethanol (v/v) with respective ethanol:water (v/v) controls. Injury was recorded every 4-8 hours for 72 hours, while plants were kept in a growth chamber at 25C (12 hour night/day cycle). Secondly, inbreds Va14 and B73 treated with 20, 25, or 30μM cercosporin concentrations dissolved in 1.64, 2.5, or 3.0% ethanol:water (v/v), respectively. Corresponding ethanol:water controls were used for each cercosporin treatment. Lesion lengths (mm) on the first and second leaf of Va14 and B73 were measured 72 hours after treatment. Prior to the preliminary studies above, Pioneer Brand 3320 was subjected to varying levels of ethanol:water at 1, 3, 7, 9, 11, 13, 15, 17, 19, or 21% (v/v) for determination of ethanol phytotoxicity.

Inbreds ranging in their degree of resistance or susceptibility to GLS in the field (susceptible: B73, B68, Mo17, and H99; more resistant: NC250, NC250a, NC264, NC268, Pa875, Va14, and Va59) were used for three shoot uptake evaluations. Shoots of fourteen-day-old seedlings were excised under water and transferred immediately into

a 20 μM cercosporin solution dissolved in 3% acetone (v/v) or a control of 3% acetone:water (v/v) for 72 hours. The first experiment consisted of a light replicate in the growth chamber (25 C) at an alternating 12 hour night/day cycle and a dark replicate enclosed in a cabinet at room temperature (22 ± 2C). A second experiment was performed in the lighted growth chamber as described above and included documentation of inbred height, width of shoot and leaf stage were prior to exposure to the lighted growth chamber environment. A third experiment consisted of a 20μM cercosporin solution dissolved in 100% acetone carrier under lighted growth chamber conditions.

Seedlings were harvested after 72 hours by sectioning necrotic and green leaf tissue. Fresh weights of samples were measured and samples were frozen and stored at -80 for three months prior to extraction for chromatographic determination of cercosporin.

Fluorescence and light microscopy

An Epifluorescence-Zeiss Fluorescence system consisting of a Zeiss (Axioskop) microscope with a epifluorescence-Zeiss fluorescence illuminator was used for bright field and fluorescence microscopy studies. The light source, a HBO 50 mercury lamp and three lens collector was used with a narrow band filter H2 which fluoresces at a 390-460nm wavelength.

Photomicrographs were produced with a Zeiss 35mm microscope camera MC100 with automatic exposure control. Ektochrome film at ASA 400 was used with 10-14 second exposure times.

Purified cercosporin crystals were suspended in distilled water and placed on a

glass microscope slide with coverslip, then veiwed at 100X magnification. Crystals were left under tungsten lighting (280nm) for 60 minutes. Microphotographs were taken at 30, 40, and 60 minutes under bright field and fluorescence in the same field of view. Fresh or air dried leaf, root, and shoot tissue were photograghed from B73 X Pa91, B68 X Va14, and Pioneer Brand 3320 pruned and/or nonpruned root uptake experiments. Injury of leaf, shoot, and root tissues were compared to respective non-treated control tissue under bright field and fluorescence. Observations were documented with microphotographs.

Pigment extraction procedures

Pigments from B73 X Pa91 and B68 X Va14 nonpruned root samples were extracted with a procedure modified from Jenns, *et al.* (1989) and Fore, *et al.* (1988). The samples were ground by a basic Omnimixer homogenizer (type OM) (Omni Corp. International, Waterberry, CT) in ethyl acetate:acetone (60:40) four times, decanting extracts every three minutes. Extracts were combined and given a low speed centrifugation at 2000 X G for one hour. Supernatants were evaporated to dryness under nitrogen gas. Samples were hydrolyzed with 1 ml of 5N KOH for five minutes, then 1 ml of 5N HCL was added. Semi-purified extracts were centrifuged at 2000 X G for ten minutes and supernatants were partitioned against chloroform. The organic phase was then collected and evaporated to dryness under nitrogen gas. Samples were resuspended in ethyl acetate and syringe filtered through 0.2μm filters. Samples were streak plated onto high performance thin layer chromotography (HPTLC) plates. Corresponding Rf

values to that of a cercosporin standard were individually scraped from the plate and respotted for qualitative analysis.

All pruned root plant samples from root uptake experiments were ground with an Omnimixer in chloroform:methanol (3:1) four times, decanting supernatants every three minutes. Samples were centrifuged at 2000 X G for one hour. Supernatants were partitioned against water and organic phase were collected. Extracts were evaporated to dryness under nitrogen gas, resuspended in 0.5ml of ethyl acetate and streak plated onto HPTLC plates. Spots with Rf values corresponding to the cercosporin standard were scraped and respotted for qualitative analysis.

Shoot uptake samples were also extracted by a procedure modified from Fore *et al.* (1988) by grinding samples with liquid nitrogen in a mortar and pestle. Ground tissue was extracted in 3:1 chloroform:methanol for 3 days, changing supernatant daily. Supernatants were combined and vaccuum filtered through Whatman No.1 filter paper. Filtered supernatants were transferred to glass tubes and partitioned against water. The organic phase was collected and syringe filtered through a 0.2µm filter. Sample was evaporated to dryness under nitrogen gas and redissolved in a 100µl ethyl acetate for which the entire sample was streaked onto HPTLC plates. Zones with Rf values corresponding to the standards for cercosporin were scrapped and respotted three times and analyzed by HPTLC and scanning densitometry.

Extraction efficiency and toxin recovery incidence

Corn seedlings (14 days old) were placed in a growth chamber at 25C and 15%

relative humidity with alternating 12 hour night/day cycles. Seedlings were treated with 34.2 μM concentrations of cercosporin dissolved in 3% acetone via shoots for 72 hours, as previously described. Plants were discarded and the remaining cercosporin solution was partitioned three times against equal volumes of chloroform. The aqueous phase was discarded. The chloroform fraction was evaporated to dryness under nitrogen gas and resuspended in 3 ml of 95% ethanol (v/v). Samples were filtered through tightly packed cotton and 100 μl of 5N HCL was added to the purple pigmented (cercosporin) solutions (liquid fraction). Cercosporin, adhering to the glass tubes was then dissolved in 5N HCL (5 ml), evaporated to dryness under nitrogen gas and resuspended in 3ml of 95% ethanol. Ethanol solutions were quantified by spectrophotometry.

Methods of detection

The 10μM cercosporin treated sample of B68 X Va14 from the nonpruned root uptake experiment, a 1.068 μg cercosporin standard, and a healthy control tissue extract was streak plated onto KC18 reverse phase thin layer chromatography (TLC) plates (Whatman International, Ltd.). Plates were developed in methanol:acetonitrile:methylene chloride:hexane (15:40:25:25 v/v/v/v).

All other HPTLC analyses were performed on pre-washed or nonwashed HP-K silica gel plates (Whatman International, Ltd.) treated with 2% phosphoric acid (H_3PO_4) and charred overnight or for 1 hour at 100C. Hexane:isopropanol (8:2 v/v) (Kuyama and Tamura, 1957) was used as a developing solvent.

A fluorescing red spot observed under long wave UV (360-480 nm) light at an Rf

value corresponding to the standards was used to identify and quantify cercosporin. Cercosporin concentrations in ng per gram of fresh weight tissue were determined by scanning densitometry. This system consisted of a CAMAG II Scanner/densitometer and a CAMAG SP4270 Integrator (CAMAG Scientific, Inc.) operating in the absorbance mode at 480 nm, with a slit width of 1.5 mm. Scanning was performed at a speed of 1mm/sec. Quantitative amounts were determined by use of a standard curve and linear regression.

Experimental design and statistics

Glasshouse experiments. The experimental design was randomized complete block (RCB) with four plants consisting of three treated veins/leaf. Seedling (21- and 39- day old) responses were designed as separate experiments. Each experiment was repeated three times and the highest and lowest measurements for each parameter was deleted prior to statistical analysis.

Growth chamber experiments. All growth chamber experiments were RCB design with three to four replicates per treatment. The highest and lowest percent injury ratings were omitted from statistical analysis.

Data analysis was performed by analysis of variance (ANOVA) and Duncan's Multiple Range Test (MRT) by using the Statistical Analysis System (SAS) (Anonymous, 1982).

RESULTS

Inbred field response. Significant ($P \leq 0.05$) differential responses among inbreds were

obtained by Duncan's MRT. Inbreds A632, B73, Va22, and Pa91 had blighting significantly higher than NC268, H99, NC250, NC250a, Va14, Mo17, Va59, and Pa875 by 9 September. Blighting scores were determined throughout the growing season for inbreds (Table 1). For all three replications, hypersensitive lesions developed in inbreds H99, Va59, and Pa875 by 16 August. Visual observation showed that Va14 characteristically exhibited reddish-brown pigmentation surrounding eliptical necrotic tissue.

Vein inoculation field study. Vein inoculation tests were analyzed separately by Duncan's MRT and significant differences were determined among hybrids at 5 and 14DAT. Total lesion lengths for Wilson Demand 110, Hytest 650A and Dekalb DK 689, consistantly appeared to exhibit sensitivity to cercosporin, regardless of plant or leaf age, while Helena HS 9663 and Pioneer Brand 3154 had limited responses (Table 2). Lesion widths greater than 2.5mm were produced on Wilson EXP 3166 (2.75 mm), Helena HS 9663 (2.5 mm), and Pioneer Brand 3192 (2.5 mm) by 14DAT at 50DAP. Plants observed for 77DAP, width greater than 2.0 were produced by Wilson Demand 110 (2.7 mm), Hytest HT 736 (2.6 mm), and Pioneer Brand 3352 (2.0 mm). Significant differences among replications were evident for all parameters used, but little or no damaged tissue ($\leq$ 1.6mm)was noticed on control treatments. ANOVA of total lesion length induced by cercosporin showed that upper and lower leaves differed significantly by a P value of $\leq$ 0.0001 at 14DAT (Table 3). Total lesion lengths for upper leaves averaged approximately 20 mm and lower leaves averaged approximately 36mm (1.8 times more

Table 1. Inbred response to gray leaf spot infection.

	Disease Severity Index (DSI) (0-5)[1]			
INBREDS	2AUG91	16AUG91	9SEP91	20SEP91
A632	0.60a[2]	2.97ab	4.50ab	5.00a
B73	0.77a	3.23a	4.43ab	4.97a
VA22	0.83a	2.53ab	4.30abc	4.93a
H93	0.70a	2.50abc	3.90a-d	4.83a
H99	0.00a	2.03bcd	3.10def	4.83a
PA91	0.53a	2.47abc	4.10abc	4.80a
NC264	0.57a	2.47abc	3.50b-e	4.47a
NC250	0.20a	1.50cd	3.10def	4.30ab
NC268	0.33a	2.03bcd	3.37cde	4.23ab
B68	0.73a	2.27a-d	3.50b-e	4.00ab
PA875	0.00a	1.70cd	2.07g	3.83ab
VA14	0.07a	1.27d	2.57efg	3.20bc
VA59	0.53a	1.73cd	2.17fg	3.30bc
NC250a	0.40a	1.80cd	2.73efg	3.27bc
MO17	0.07a	1.21d	2.38fg	2.56c
LSD (0.05) =	1.01	0.96	0.86	1.03
Standard dev.=	0.600	0.573	0.512	0.616
CV =	142.0	27.13	15.44	14.78

[1]Disease severity index: 0=no gray leaf spot lesions; 1=trace of lesions below ear, none above; 2=many lesions below ear, trace above; 3=severe lesion development below ear, all leaves above with lesions; 4=all leaves with severe lesion development, but green tissue visible; 5=all leaves dry and dead.

[2]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 2. Total lesion length (mm) induced by cercosporin on upper and lower leaves 5 and 14 days after treatment (DAT).

Leaf position/age	Lower leaves (50 DAP[1])			Upper leaves (77 DAP)	
HYBRID	5DAT	14DAT[2]	HYBRID	5DAT	14DAT
Wilson Demand 110	49.94a[3]	61.69a	Wilson Demand 110	36.0a	32.75a
Wilson 185	36.75ab	48.63ab	Hytest HT 736	30.13abc	29.19ab
Hytest HT X7809	31.53ab	48.30ab	Pioneer Brand 3140	30.25ab	26.313abc
Augusta P560	35.00ab	46.63ab	Hytest HT 650A	22.88b-e	25.38abc
Helena HS 9663	33.18ab	45.50ab	Dekalb DK 689	13.38def	25.00abc
Dekalb DK 689	28.25ab	42.00ab	Pioneer Brand 3192	25.00bcd	24.63abc
Hytest HT 736	38.57ab	40.88ab	Augusta 614	18.75b-e	23.88abc
Helena HS 97	31.88ab	39.88ab	Auguata P560	20.81b-e	22.55a-d
Hytest HT 650A	26.25ab	39.31ab	Pioneer Brand 3352	22.81b-e	22.25a-d
Pioneer Brand 3154	15.69ab	39.31ab	Helena HS 9911	23.88bcd	21.44a-d
Augusta 614	35.06ab	38.13ab	Wilson 1852	18.06de	20.50a-d
Wilson EXP 3166	27.19ab	37.81ab	Pioneer Brand 3136	18.54cde	20.36a-d
Helena HS 9773	32.94ab	32.00ab	Helena HS 9773	21.75b-e	19.19bcd
Pioneer Brand 3140	17.00ab	30.13ab	Augusta 505	19.06b-e	18.13b-e
Augusta 505	17.75ab	29.31ab	Pioneer Brand 3320	21.31b-e	17.75b-e
Pioneer Brand 3136	29.44ab	28.31ab	Helena Hs 97	21.75b-e	17.75b-e
Pioneer Brand 3320	16.63ab	27.19ab	Hytest HT X7809	21.5b-e	14.75cde
Helena 9911	21.56ab	27.19ab	Wilson EXP 3166	14.25def	13.75cde
Pioneer Brand 3352	20.88ab	24.81ab	Helena HS 9663	11.62ef	9.94de
Pioneer Brand 3192	12.31b	9.81b	Pioneer Brand 3154	6.79f	6.21e
LSD=	28.15	36.22		9.75	11.09
Standard deviation=	19.91	25.61		6.89	7.84
CV=	72.32	69.52		33.01	39.11
Average length of 50% ethanol lesion	1.5mm	1.5mm		1.0mm	1.1mm

[1]Days after planting.
[2]Data are listed from the most to the least sensitive by 14DAT response to cercosporin.
[3]Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

Table 3. ANOVA of total lesion length (mm) induced by cercosporin measured at 14 days after treatment (DAT) for upper and lower leaves.

Source of variation	df	F value	P value
Leaves (upper/lower)	1	44.96	0.0001
Hybrid	19	1.33	0.2038
Block (replication)	3	12.91	0.0001
Leaf * hybrid	19	1.05	0.4262
Leaf * block	3	11.07	0.0001
Hybrid * block	57	1.22	0.2243
R^2=0.82			
CV=56.2			

toxin injury on lower than upper leaves). However, plant age (50 or 77 DAP) may have had an affect on lesion size. Total lesion length significantly increased between 5 and 14 DAT. Significant differences ($P \leq 0.0001$) were found between upward and downward extensions of lesions at 14 DAT. Upward extension of lesions extended toward the leaf tip were 1.4-1.5 times more than those extended back toward the stalk.

Glasshouse vein inoculation study. Glasshouse vein inoculation trials showed that upward and downward extensions were not significantly different between hybrids for 21- or 39-day old plants. However, ANOVA of width (mm) of cercosporin induced lesions at 8 DAT showed that widths differed significantly at $P \leq 0.0001$ and were wider (1.5X) than those of 21-day old plants and that hybrid responses showed mild significance ($P \leq 0.0441$) (Table 4, Figure 1.). Younger seedling plants appear to be more sensitive to the toxin than older plants. ANOVA of cercosporin lesion length also showed a mildly significant correlation ($P \leq 0.0282$) between 21- and 39-day old plants (Table 5). Lesion length for 21- day old plants were longer than those of 39- day old plants (Figure 2).

Table 4. Analysis of variance of width of cercosporin induced lesions on 21 and 39 day old plants at 8 DAT.

Source of variation	df	Mean square	F value	P value
Experiments	2	0.0974	0.47	0.6286
Age (21 or 39 days old)	1	6.5402	31.34	0.0001
Hybrid	3	0.5842	2.80	0.0441
replications	7	0.1939	0.93	0.4877
Experiments * hybrid	6	0.0911	0.44	0.8528
Experiments * replication	14	0.2298	1.10	0.3668
Hybrid * age	3	0.2960	1.42	0.2420
Error	98	0.2087		
R^2=0.65				
CV=50.8				

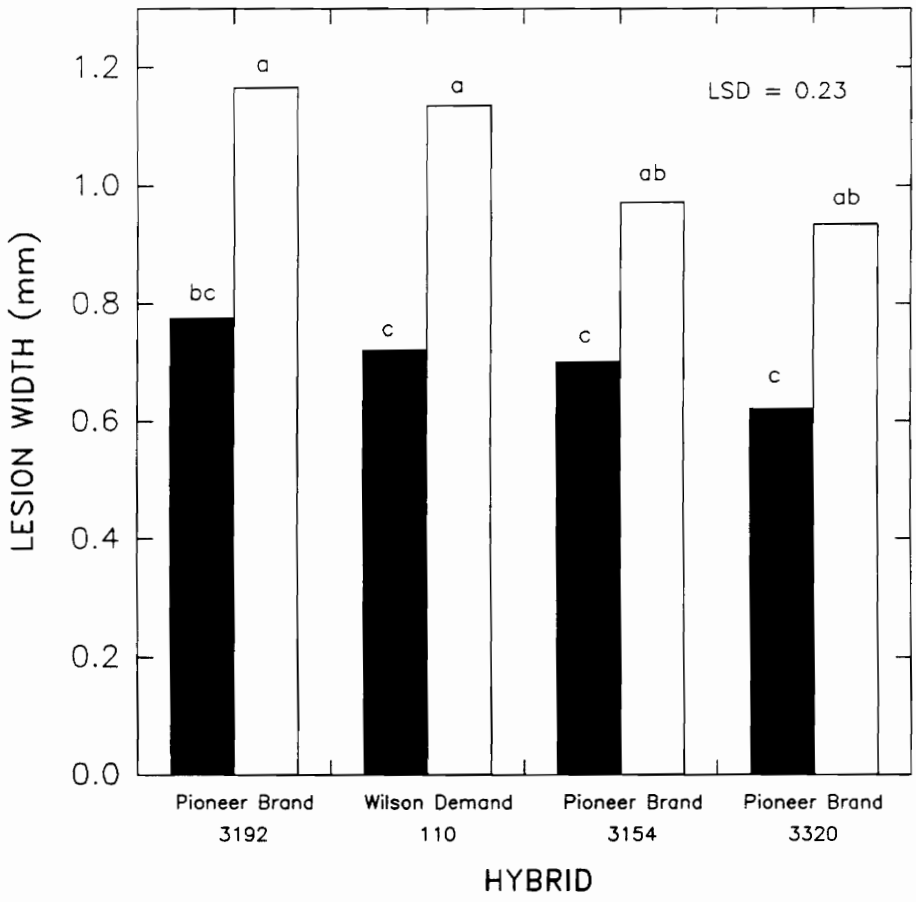


Figure 1. Average width of cercosporin induced lesion on hybrids at 21-day old (as denoted by a solid bar) and 39-day old (as denoted by an open bar) plants. Means with letters in common do not differ significantly (P=0.05) by Duncan's MRT.

Table 5. Analysis of variance of total lesion length induced by cercosporin on 21 and 39 day old plants at 8 DAT.

Source of variation	df	Mean square	F value	P value
Experiments	2	2349.82	84.76	0.0001
Age (21 and 39 days old)	1	138.27	4.99	0.0282
Hybrid	3	23.46	0.85	0.4724
Replication	7	4.46	1.61	0.1446
Experiment * Hybrid	6	51.57	1.86	0.0972
Experiment * replication	14	42.69	1.54	0.1148
Hybrid * age	3	18.10	0.65	0.5832
Error	84	27.70		
R^2=0.79				
CV=40.84				

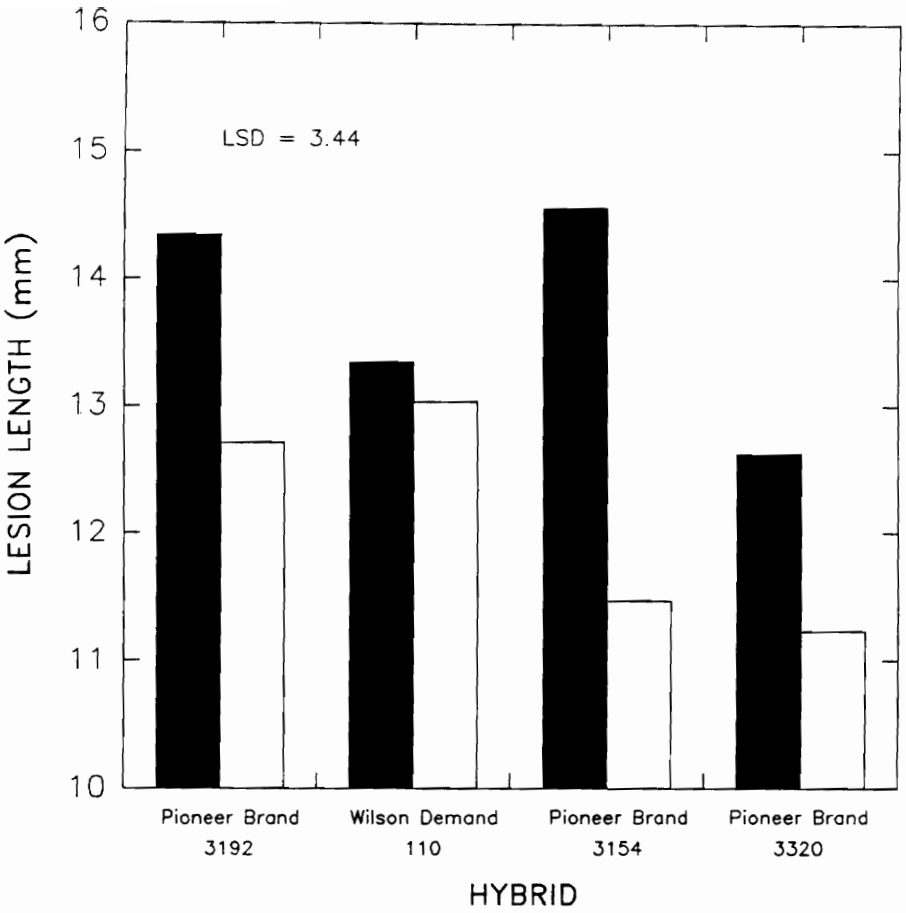
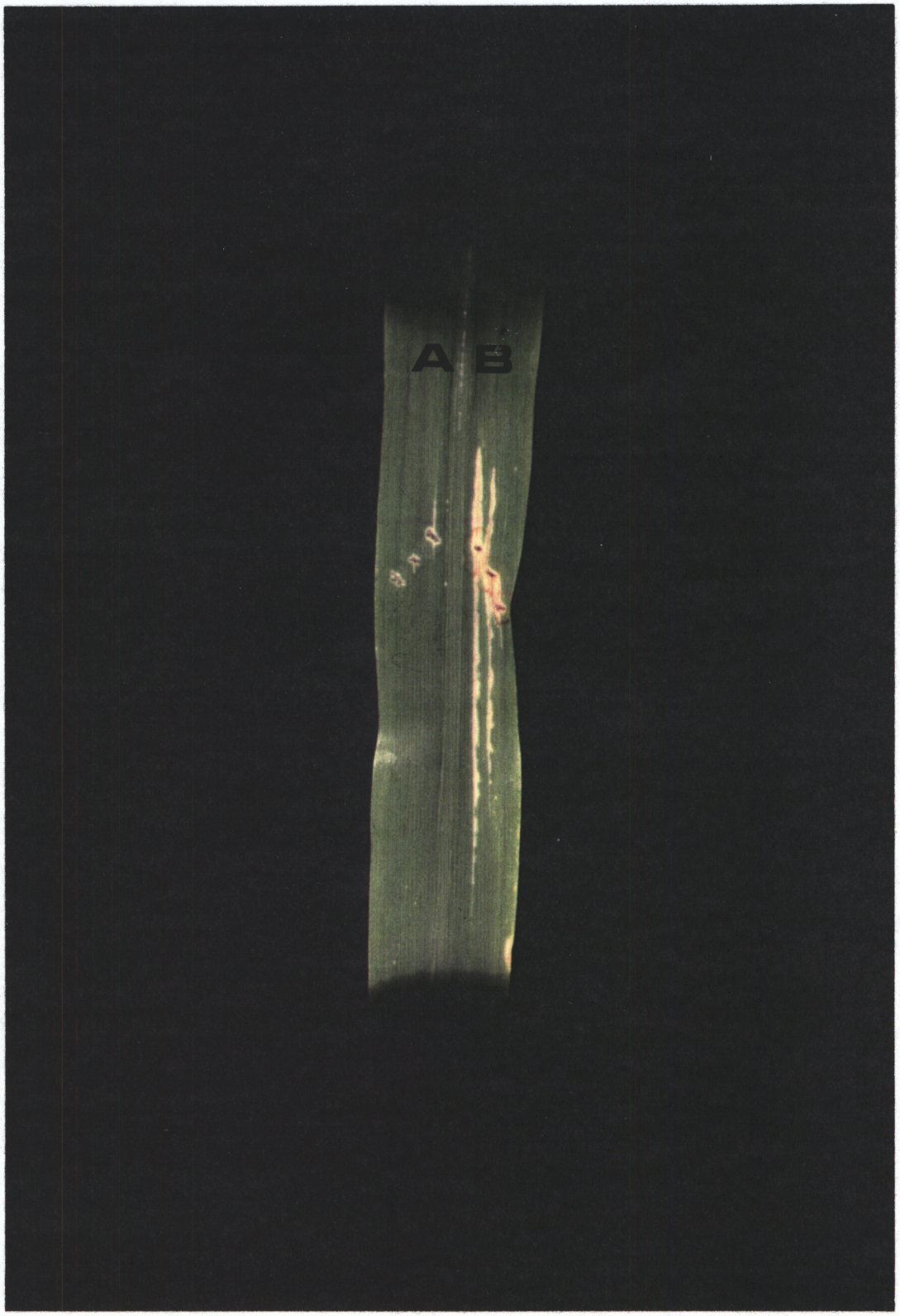


Figure 2. Total length of cercosporin induced lesions on hybrids at 21-day old (as denoted by a solid bar) and 39-day old (as denoted by an open bar) plants.

However, differential responses between hybrids could not be determined. Significant differences between lesion length induced by cercosporin were evident for inner verses outer tertiary veins. The vein closest to the midvein consistantly resulted in longer than lesions those near the leaf edge (Color Plate 2).

Root uptake experiments. Visual observation of plants subjected to root uptake of cercosporin treatment on nonpruned roots showed that reddening of tertiary veins and midvein of the first vegetative leaf were exhibited in the treated plants of B73 X Pa91 and B68 X Va14. Redness of these veins extended over 3/4 of the leaf. This which was much more predominant in B68 X Va14 seedlings than in B73 X Pa91 and more intense reddening occurred in the 50μM cercosporin treatments. Greater tip injury resulted from the 10μM cercosporin treatment. B68 X Va14 and B73 X Pa91 controls showed slight reddening of the midvein and tertiary veins, however, this reddening was only apparent on the underside of the leaf and extended over only 1/4 of the leaf. Observations of symptoms pruned seedlings illustrated the same reddening as mentioned for nonpruned roots, except that no red venation was observed on the first vegetative leaves of B73 X Pa91. Pruned root seedlings treated with 10 and 50μM cercosporin treatments exhibited watersoaked tips by 48 hours. Injury on B73 X Pa91 and B68 X Va14 appeared on all treatments, except the 5 μM cercosporin concentration which remained symptomless for pruned and nonpruned root seedlings at 48 hours. Eliptical lesions surrounded by a chlorotic halo, characteristic of early GLS lesions, appeared on the first, second, and third vegetative leaf tip of B73 X PA91, B68 X VA14, and B73 after 48 hours for the

Color plate 2. Vein inoculation of Pioneer Brand 3320 at 21 days of age. The side of the leaf treated with 50% ehthanol is denoted by "A", and 1.2 μM cercosporin treatment is denoted by "B".


A B

10μM cercosporin treatments. Root injury was more severe with increased concentrations of cercosporin solution while roots from ethanol treatments appeared healthy. High cercosporin concentrations also exhibited more severe injury than their ethanol controls.

Shoot and leaf tissue of B73 X Pa91 and B68 X Va14 from nonpruned root seedlings had no detectable cercosporin. However, leaf tissue from nonpruned root seedlings exhibited a red fluorescent band at Rf = 0.39 for B73 X Pa91 and Rf = 0.36 for B68 X Va14 with a 50μM cercosporin treatment believed to be cercosporin. A 0.534μg cercosporin standard had an Rf = 0.36.

Pioneer Brand 3320 was used for preliminary root uptake in light and dark experiments. Percent injury was estimated on a scale from 0-9 described previously for use on seedling germplasm treated with cercosporin treated through roots or shoots. For the lighted experiment, an average of 81.7% plant injury occurred after 96 hours exposure to a 50μM cercosporin treatment and 90% plant injury after treatment with 100μM cercosporin solution. Seedling injury produced in darkness with the 100μM cercosporin treatment was not significantly different than the 50% ethanol:water (v/v) control. No injury was noted for any cercosporin treatments kept in the dark. Pioneer Brand 3320 leaf, root, and shoot tissue was quantitatively analyzed by HPTLC for the presence of cercosporin. Cercosporin was positively identified in all root tissue. In the lighted experiment, cercosporin was identified from shoot tissue of the 100μM (Rf = 0.28) and leaf tissue of 50 μM (Rf = 0.302) cercosporin treatments with 1.068 μg standards at Rf = 0.25 and 0.32, respectively. No cercosporin was positively identified

from seedling tissue kept in the dark.

Light and fluorescence microscopy. Leaf, shoot, and root tissue of cercosporin and ethanol treated seedlings from preliminary root uptake experiments were examined by light and fluorescence microscopy. Round, yellow, fluorescing crystals were observed from pruned root tissue of B73 X Pa91 and B68 X Va14 with 5, 10, and 50 μM cercosporin treatments. B68 X Va14 contained crystals that fluoresced in the necrotic leaf tissue of the 50μM cercosporin treatment. The 10μM cercosporin treatment had yellow fluorescing crystals within intercellular spaces of necrotic leaf tissue. Pioneer Brand 3320 seedling tissue contained crystals in 50 and 100μM treated roots (Color Plate 3d) and necrotic leaf tissue of 50 and 100μM cercosporin treatments. No crystals were found in shoot tissue nor in ethanol control tissue.

Purified cercosporin crystals were red in color under bright field (Color plate 3a) at zero minutes. However, when crystals were left under tungsten light for 30 minutes they fluoresed yellow (Color plate 3b). As time increased to 45 minutes, more of the purified cercosporin fluoresced yellow (Color plate 3c). Still more of the cercosporin crystals fluoresced with longer exposure times to tungsten light.

Necrotic leaf tip extracts from Pioneer Brand 3320 and B73 were qualitatively identified at an Rf = 0.30, corresponding to a 1.068 μg standard. Quantification was performed by scanning densitometry. Linear regression analysis of the standard curve had an R^2 = 0.99. Treatment concentrations for each test plant with cercosporin recovery are shown in Table 6. Cercosporin was identified at an Rf = 0.30 in the B73

Color plate 3. These microphotographs are purified cercosporin crystals under bright view (as denoted by A), crystals after exposure to bright view for 30 minutes then fluoresced (as denoted by B), crystals after exposure to bright view for 45 minutes then fluoresced (as denoted by C), and root tissue from 50 μM cercosporin treated roots of Pioneer Brand 3320 fluoresced (as denoted by D).

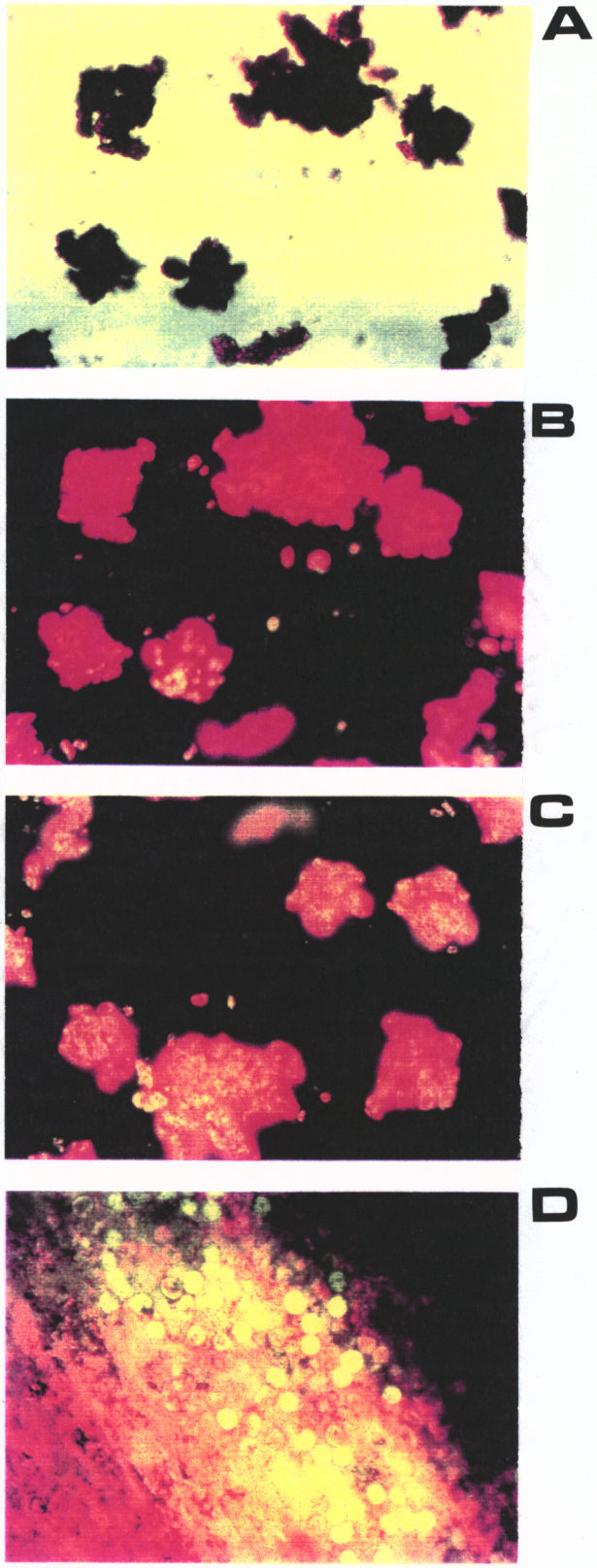

A
B
C
D

Table 6. Recovery of cercosporin (ng/g of tissue) from necrotic tissue of Pioneer Brand 3320 and B73 tissue.

Germplasm	Cercosporin treatment (μM)	ng cercosporin/g of tissue
Pioneer Brand 3320	15	951.0
	25	252.8
	35	1114.0
	45	1437.0
B73	15	1090.0
	25	+[1]
	35	265.9
	45	511.2

[1] "+" denotes positive identification of cercosporin.

sample treated with 25μM of cercosporin solution. However, quantification could not be performed on this sample because the scanning densitometer indicated that output was out of range. Percent injury of Pioneer Brand 3320 verses B73 was 83.3 and 10.4, respectively at the 25 μM cercosporin treatment. First symptoms of injury occurred between 28 and 38 hours after treatment. Ethanol controls (ranging from 1.6-4.8% v/v) caused little or no injury, except for 4.8% ethanol (v/v). From the preliminary ethanol phytotoxicity experiment, visual ratings showed that any concentration of ethanol greater than 3% caused severe damage to seedling tissue.

Shoot uptake experiments. Va14 and B73 were used to determine if differential responses between resistant and susceptible germplasm to GLS could be detected by shoot uptake of cercosporin. Symptoms first appeared 36 hours after exposure of seedlings to toxin solutions. Treatments of 20 and 25μM delivered significant ($P \leq 0.05$) injury over the

control for both B73 and VA14 (Color plate 4). However, 3% ethanol used with 30μM cercosporin solution still appeared to induce slight damage to control seedlings. Cercosporin concentrations of 20, 25, and 30 μM produced distinctive injury responses. Characteristic necrotic eliptical lesions surrounded by a yellow halo appeared on both vegetative leaves of B73 and Va14, along with necrotic tip injury surrounded by a yellow border (Color Plate 5). However, differential responses between inbreds were not significant. The injury produced on the first leaf was significantly higher than lesions measured on the second leaf. The average lesion on the first leaf measured 43.3 mm for B73 and 31.7mm for VA14. However, the lesion lengths for the first leaf can not be attributed solely to cercosporin, since ethanol control lesion lengths were not significantly different. The lesion on the second leaf averaged 14.0 mm for both inbreds. No significant differences occurred between lesion lengths for these two inbreds.

Comparisons of shoot uptake experiments under dark verses light treatments showed considerably less injury produced by cercosporin in darkness (Table 7). NC264 and Pa875 seem to be the most sensitive to cercosporin in absense of light at 31.7 and 35.0 % plant injury, respectively. Evaluations for shoot uptake resulted in significant ($P \leq 0.0001$) variations between replicated experiments using 20 μM cercosporin treatments (Table 7). However, the 3 % acetone carrier caused minimal injury for both replications. The reason for variability among germplasm was speculated to be caused by differences in vigor among inbreds, thus, inbred size was measured by the width of shoot just above

Color plate 4. Inbreds, B73 and Va14, after a 92 hours of treatment with 25 μM cercosporin solution dissolved in 2.5% ethanol (v/v) and a 2.5% ethanol (v/v) control applied via shoots. Ratings resulted in 78% plant injury on treated seedling (as denoted by A) and 20% injury on control seedling (as denoted by B) as compared to Va14 with 82% plant injury on treated seedling (as denoted by C) and 0% plant injury on its control (as denoted by D).

D
C
B
A

Color plate 5. Comparison of a cercosporin induced lesion (as denoted by A) on B73 to early season GLS lesions in the field (as denoted by B).

B
A

Table 7. Summary of germplasm response to cercosporin shoot uptake experiments.

	Experiment I[1]		Experiment II[2]			
	Light	Dark	Light			
Germplasm	Percent Plant Injury (0-100)			width	height	leaf stage
NC264	80.0a	31.7ab	82.5a	3.08a	17.5b	2.7bc
NC250a	58.3b	7.3def	82.5a	3.02a	17.28bc	3.0bc
B73	46.7bc[3]	8.0def	78.8ab	2.78a	19.35ab	2.7bc
NC250	46.7bc	15.7cd	63.8c	3.12a	12.88cd	3.0bc
NC268	41.7cd	7.3def	67.5c	3.12a	19.7ab	3.8a
Pa875	30.0de	35.0a	47.5d	3.05a	12.73d	2.7bc
Va14	26.7de	23.3bc	70.0bc	3.05a	15.35bcd	3.2b
Mo17	20.0e-f	15.0cde	48.8d	3.18a	15.87bcd	2.7bc
H99	25.0ef	13.3c-f	36.3e	3.05a	15.35bcd	3.2b
Va59	18.3e-h	4.7def	31.3e	3.32a	16.92bcd	2.5c
B68	*[4]		20.0f	2.75a	15.5bcd	2.8bc
LSD=	14.5	9.70	9.9	0.60	3.97	0.5
sd=	8.79	5.89	6.97	0.51	3.41	0.40
CV=	33.77	54.13	21.84	16.17	20.12	13.80

Little to no injury was produced by the 3% acetone:water (v/v) control.

[1] Shoot uptake of a 20 µM cercosporin solution in 3% acetone performed under growth chamber environment and at room temperature in the dark.

[2] Shoot uptake of a 20 µM cercosporin solutions in 3% acetone performed in the light and measurements of inbred width (just above the soil line), height, and leaf stage were collected.

[3] Means with letters in common do not differ significantly ($P \leq 0.05$) by Duncan's MRT.

[4] "*" denotes that inbred B68 was not included in experiment I.

the soil line, height, and leaf stage. These parameters were averaged between replicates for the second shoot uptake experiment (Table 7). Measurements appeared to indicate that inbred width and leaf stage (ranging from 2 to 4) were not positively correlated to percent injury. However, some taller plants appeared to injured more than shorter plants (Table 7). These parameters were not constant for any one inbred throughout a replicated experiments.

Of the 11 inbreds evaluated for sensitivity to cercosporin by shoot-uptake, only five, B73, H99, Va59, NC250a, and NC264 gave consistant results over the two experiments. H99 and Va59 were significantly more sensitive to cercosporin ($P \leq 0.05$, Figure 3). H99 seedlings averaged 31.7% plant injury and NC250a averaged 72.5% (Figure 3). By comparison, the greatest injury of replicates of H99 (68%) was compared to a representitive NC250a replicate (88%) (Color plate 6). Injury to control plants was minimal; however, some inbreds were more sensitive to acetone treatment than others. For instance, 8.8% of H99 resulted in necrotic tip injury on three leaves while Va59, NC250a, and NC264 only had two leaves with yellow to necrotic tips.

Inbreds exposed to a 20μM cercosporin solution dissolved in 3% acetone were quantitatively analyzed to determine the amount of cercosporin present in necrotic leaf tissue. The standard curve had an $R^2 = 0.96$. The amount of cercosporin recovery ranged from 407.1-1076.7 ng/g of tissue in inbreds, H99, Pa875, Va14, NC250a, and Mo17 (Table 8). No cercosporin was recovered from control plants. H99 and NC250a germplasm responses measured as percent plant injury, (Figure 3), showed that NC250a

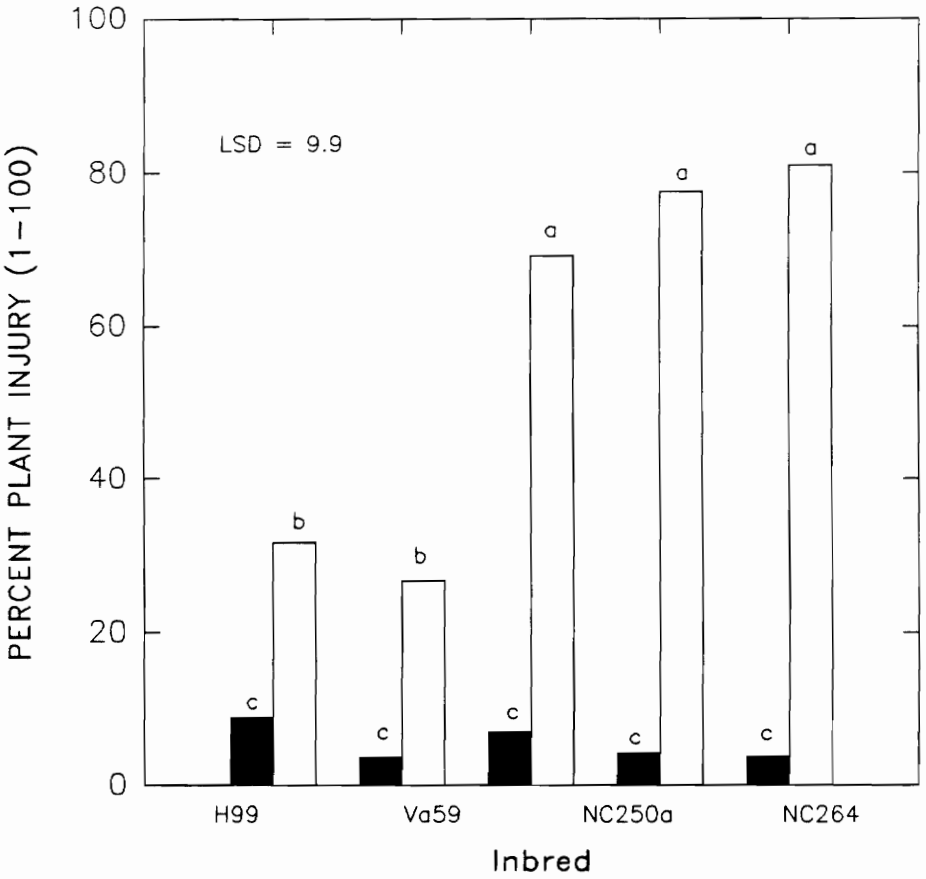


Figure 3. Combined percent injury of inbred germplasm response to 20uM cercosporin dissolved in 3.0 % acetone (as denoted by open bar) and 3.0 % acetone control (as denoted by solid bar).

Color plate 6. Comparison of individual seedlings of H99 and NC250a after 72 hours exposure to a 20μM cercosporin treatment dissolved in 3% acetone applied via shoots. H99 resulted in 68% plant injury (as denoted by A) as compared to NC250a with 88% (as denoted by B).

B
A

Table 8. Cercosporin recovery (ng/g of tissue) from necrotic tissue of inbred germplasm.

Germplasm	20 µM cercosporin treatment in a 100% acetone carrier	20 µM cercosporin treatment in 3.0 % acetone carrier (ng cercosporin/g)
B73	+[1]	–[2]
H99	+	430.7
NC250	+	–
NC264	+	–
NC268	+	–
B68	+	–
Pa875	+	1076.7
Va14	+	680.3
Va59	+	–
NC250a	+	423.2
Mo17	+	407.1

[1] "+" denotes positive identification of cercosporin by qualitative HPTLC analysis.
[2] "–" denotes no detectable cercosporin.

resulted in more plant injury than H99 with similar cercosporin recovery from necrotic leaf tissue. Cercosporin (260.0 ng/g) was recovered from non-injured leaf tips of only one toxin treated inbred, NC250a, exposed to darkness. Cercosporin recovery from only one inbred may have resulted because the plants were not exposed to low relative humidity to favor transpiration as were those exposed to light.

The shoot uptake experiment exposing seedlings to 20µM cercosporin solution dissolved in 100% acetone turned seedlings white. All inbreds tested resulted in greater than 69.3% (Mo17 and Pa875) plant injury (Table 7). The first leaf or entire plant seedling of all inbreds tested, showed a watersoaked appearance after 12 hours exposure in darkness. After 24-36 hours, green leaf color dissappeared and reddening of all midveins and tertiary veins was observed. Leaves turned necrotic after 72 hours exposure under growth chamber environment. Pigments were extracted from leaf tissue over a

three day period by the procedure modified from Fore *et al.* (1988). All inbreds showed a red spot (Rf = 0.30) which fluoresced under long wave ultraviolet light and corresponded to the purified cercosporin standard. No spots were observed from control extracts at the corresponding Rf value of the standard, which was further substantiatedby scanning densitometry.

Extraction efficency and toxin recovery. Initially, glass tubes contained 18.23μg/ml of a cercosporin solution. Spectrophotometric analysis of tubes containing seedlings averaged of 7.29μg/ml of cercosporin still in solution (liquid fraction). However, 7.0μg/ml of cercosporin was recovered from glass tubes (glass fraction). This left 3.9μg/ml of unaccountable cercosporin. In tubes containing cercosporin (18.23μg/ml) alone, 9.1μg/ml and 2.62μg/ml of cercosporin was recovered from the liquid and glass fraction respectively. It was noted that two partitions against chloroform do not remove all the cercosporin from the water phase. The water phase was colorless after three or more partitions.

DISCUSSION

The inbred response to blighting by GLS disease under natural field conditions confirmed work done by Thompson, *et al.* (1987) where parents were rated for their degree of susceptiblity or resistance, *ie.*, N28rhm, B73rhm, B73, B73ARB, H99, NC250, Va59, and T234. Ayers et al. (1984) and Huff et al. (1988) also predicted that Pa875, Va59 and B68 were more resistant and H93 susceptible to GLS.

Vein inoculation studies. Field vein inoculation study showed that lesion length on upper

and lower leaves of a plant are significantly different; however, upper and lower leaves also differed in age by 27 days. Therefore, whether lesion responses were due to leaf position or plant age was unclear. Upward and downward extensions of lesions on treated plants, even though significantly different, were not indicative of GLS disease response in the field. No differential responses between hybrids (Table 3) could be determined because of block variation. Part of the significant block effect could be attributed to having different persons administering treatments. Therefore, use of vein inoculation as a method of evaluating germplasm was not very sensitive and was highly variable.

Less variation occurred in glasshouse evaluations of vein inoculation studies and results suggested that 39-day old plants were more sensitive to the toxin than 21-day old plants. Cercosporin appeared to move into the parenchyma cells of the older plants more readily than in younger plants. A parellel between seedlings and mature plants cannot be made. It is suggested that plant and/or leaf age may be important contributions to the role of cercosporin in the epidemiology of GLS disease. Gwinn, *et al.* (1987) studied the effect of cercosporin on corn plant age at 1-, 2-, and 3-month old with leaf discs emersed in 1.2μM cercosporin in 2% aqueous methanol and concluded that older tissue was more sensitive than youger tissue. By using the vien inoculation method under well monitored glasshouse conditions, further experimentation may determine the effect of cercosporin on mature plants and leaf age. Field experimentation would be too variable, since factors such as fluctuating environmental conditions and canopy effect may interfere. However,

reproducing conditions present in the field, such as canopy effect, where older leaves receive less light than upper leaves may produce useful results.

Root uptake experiments. More red venation was observed on a more resistant hybrid than a susceptible hybrid in response to cercosporin treatment. This reddening was apparent in control plants and became more prevelant after cercosporin treatment. Greater reddening occurred in seedlings treated with high concentrations of cercosporin further suggesting that cercosporin may have induced these responses. Thus, this may suggest that greater amounts of anthocyanin or carotenoids were produced in resistant plants as a plant-induced resistance response to cercosporin treatment. Typically under field conditions, inbred Va14 produces red borders surrounding the GLS lesions as has been observed in other more resistant hybrids (Stromberg and Donahue, unpublished). This suggests that a pigment such as anthocyanin or carotenoids may play a role in the resistance response in the field. Results indicated that cercosporin may trigger that response.

Greater plant injury occurred from cercosporin treatment of pruned than nonpruned roots. No cercosporin was identified in leaves or shoots of tissue from nonpruned root experiments. This result may be because cercosporin may become bound to tissues before it could pass through the root. Alternatively, lack of toxin recovery may have been a result of an inadequate extraction procedure, *ie.*, modified from Fore *et al.* (1988) and Jenns *et al.* (1989).

Detection of cercosporin from nonpruned root studies resulted in tentative

identification of cercosporin from necrotic leaf tissue. Fluorescence microscopy results suggested that yellow fluorescing crystals found in the leaf and root tissue of cercosporin treated seedlings may be cercosporin or a cercosporin derivative. Fluorescence microscopy of purified cercosporin crystals supports the hypothesis that cercosporin may be metabolized or altered within plant tissue. More experimentation needs to be done to determine whether this yellow fluorescing form is a derivative of cercosporin.

Shoot uptake experiments. Eliptical necrotic lesions characteristically surrounded by a yellow halo were associated with cercosporin treated plants in both root and shoot uptake experiments. Shoot uptake experiments showed that cercosporin treatments exhibited this characteristic response and the amount of cercosporin in tissue was quantified. Overall, germplasm responses were not consistant among experiments. However, when comparing the five inbreds whose responses were not significantly different, NC250a showed greater plant injury than H99 with similar amounts of cercosporin recovered from necrotic tissue. When comparing these results to the inbred field trial H99, Va59, NC250a, and NC264 germplasm responses to cercosporin were not indicative of their reaction to *C. zeae-maydis* in the field. However, B73 was consistantly more sensitive to cercosporin and was susceptible to *C. zeae-maydis*. This suggests that some germplasm may be more sensitive to cercosporin than others. Interestingly, H99 and VA59 germplasm reacted sensitively to cercosporin and exhibited hypersensitive responses to *C.zeae-maydis* in the field. Reasons for this correlation between germplasm hypersensitivity to disease and sensitivity to cercosporin were confounded.

Methods of detection. The most efficient extraction procedure used was modified from Fore, *et al.* (1988) for extracting in chloroform:methanol (3:1) over a three day period changing extracts daily. This modified procedure was tested to determine how much cercosporin could have been taken up by the seedlings and/or lost in the extraction procedure (unaccountable cercosporin). From tubes containing cercosporin and corn seedling, 3.9 µg/ml of cercosporin was unaccountable. Conversely, 6.51 µg/ml of cercosporin was unaccounted for in tubes containing cercosporin alone. Since tubes containing cercosporin alone required more than three partitions to solubalize all cercosporin into the organic phase, this inconsistancy can be explained by inadequate partitions of water against chloroform. This implies that tubes consisting of both cercosporin and seedlings had less remaining cercosporin than that of cercosporin alone.

Also, between 38.4 and 49.9% cercosporin was recovered from the glassware. Therefore, some of the cercosporin present in tissue extracts could have adhered to the glassware during extraction procedures. This could account for the lack of cercosporin recovery for some germplasm (Table 7). Alternatively, it is hypothesized that the diameter of the vascular elements may have differed between inbred germplasm and also, between older and younger plants, thus accounting for some experimental variability.

CONCLUSIONS

At this point, vein inoculation and root and shoot uptake procedures were inconsistant methods for evaluating germplasm reponse to cercosporin, however, the results were capable of showing some interesting differential responses. Differences in

plant or leaf age and germplasm vessel elements is believed to cause significant experimental variation. It is postulated that larger verses smaller vein or vessel width allows for easier transport of cercosporin and may produce more plant injury when treated. Germplasm responses to cercosporin, as determined by these methods, were not indicative of germplasm responses to GLS in the field. However, repeated experimentation may show whether this technique is useful for detection of other mechanisms such as; higher carotenoids, anthocyanin and/or superoxide dismutase levels in germplasm exposed to cercosporin.

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SUMMARY

Substantial yield loss caused by GLS occurred at two locations over three years, regardless of environmental conditions. However, environment conditions may have had a large affect on the epidemiology of GLS. Less blighting occurred under more dry than wet conditions. Dry weather conditions may have negatively affected spore survival thus reducing inoculum available for further GLS development. While more moist seasons increased GLS, they also increased hybrid vigor and yield potential. To the corn producer, these occurences mask the negative effect of GLS on yield. Despite variation in climatic conditions, resistant hybrids consistantly had lower disease severities and higher yields. Grain yields of more resistant hybrids were near or over 1.5 times that of susceptible hybrids. As much as 84% of the variability in grain reduction can be attributed to the degree of blighting caused by CZM.

Correlations with blighting, yield, and hybrid resistance were further confirmed by applications of triazole and benzimidozole fungicides to control of GLS. Use of these fungicides significantly reduced blighting and increased yield as much as 1.3 to 2.2 times over the nontreated check. Reduction in blighting not only increased yield, but it also increased kernel weight. The effect of kernel weight on yield was estimated at 57 and 92%, in 1990 and 1991, respectively. During periods of hot and dry weather, GLS disease pressure was reduced, and both may have interfered with kernel fill. Blighting alone causes 76-81% loss in grain fill. Therefore, less blighting and higher yields

resulted not only from the use of fungicides, but also from the use of more resistant hybrids.

Fungicide use for cash grain corn production in Virginia is not an economically practical means to control GLS. Although, 1-2 applications of systemic triazole or benzimidozole fungicides may be beneficial for seed corn production fields, particularly, if the female parent is susceptible to GLS. Climatic conditions of the midwest are not always conducive to GLS disease, however, in areas where minimum tillage and continuous corn practices are common and when such practices are conducted under center-pivot irrigation, GLS disease severity can be yield limiting. Fortunately, use of fungicides on a susceptible hybrid increased grain yield and weight even under conditions less conducive to GLS.

Current procedures for evaluating the effect of blighting on yield have been conducted in the field employing a Disease Severity Index (DSI) to quantify disease severity. This index was the most reliable predictor of blighting on yield and grain weight when plants were scored during mid-August to early-September. Alternatively, percentage ear leaf area blighted may be a better method for determination of GLS late-season effect on yield. These methods are not limited to determining blighting effect on yield, they are also useful in identifying the action of resistance gene(s) within germplasm.

Evaluating corn germplasm for its response to the toxin, cercosporin, may aid in the identification of resistant germplasm. Screening germplasm by vein inoculation was

not useful in eliciting differential responses to cercosporin, but may be a valuable research tool to study the role of cercosporin in disease development. With this techique, 39-day old seedlings were found to be more sensitive to cercosporin than 21-day old seedlings. Cercosporin appears to move into the parenchyma cells surrounding the vessel elements of older plants more readily than those of younger plants.

By fluorescence microscopy, yellow fluorescing crystals were found associated with necrotic tissue of inbreds exposed to cercosporin via root uptake. Purified cercosporin crystals also fluoresce yellow after a period of exposure to bright light, thus suggesting that those crystals found in necrotic leaf tissue may be cercosporin. Root and shoot uptake of the toxin produced lesions on inbred germplasm that resemble those produced by CZM in the field and significantly more injury occurred to toxin treated inbreds exposed to light than to darkness.

When comparing plants subjected to cercosporin by shoot uptake, 407.1-1076.7 ng of cercosporin/g of tissue was recovered from some leaf lesion extracts of plant tissue exposed to light. However, of the plants exposed to darkness, 260 ng of cercosporin/g of tissue was recovered from noninjured leaf tips of only one toxin treated inbred (NC250a).

Shoot uptake of cercosporin by corn seedlings was an indicator of differential responses between inbreds, but was not always consistant. Only some germplasm showed consistant differential responses to cercosporin and a differential response to the same concentration of cercosporin suggesting that some germplasm were more sensitive to

cercosporin than others. Variability in the shoot uptake method may result from the inability to deliver an equal concentration of cercosporin to each plant. Germplasm responses to cercosporin, as determined by these methods, were not directly indicative of germplasm response to CZM in the field. However, these observations may lead to further investigation.

Appendix A

Average weekly temperatures for Montgomery and Wythe Counties, Virginia specified by years.

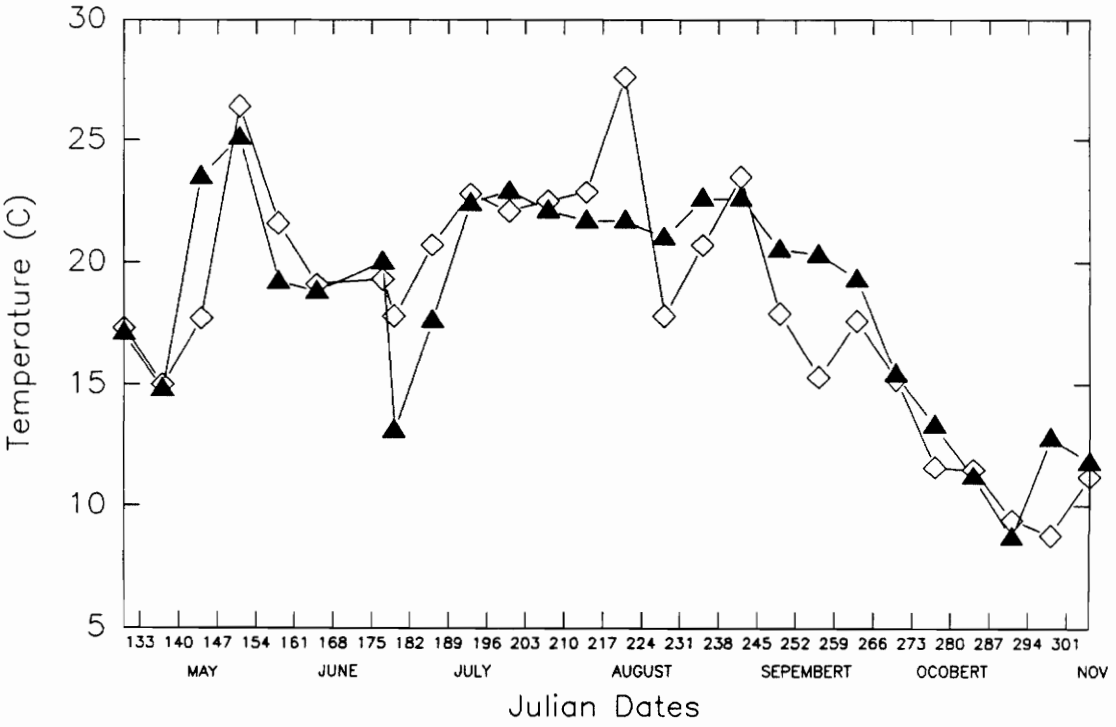

Figure A.1. Weekly average temperatures for 1989 at Montgomery (filled triangles) and Wythe (open diamonds) Counties, Virginia.

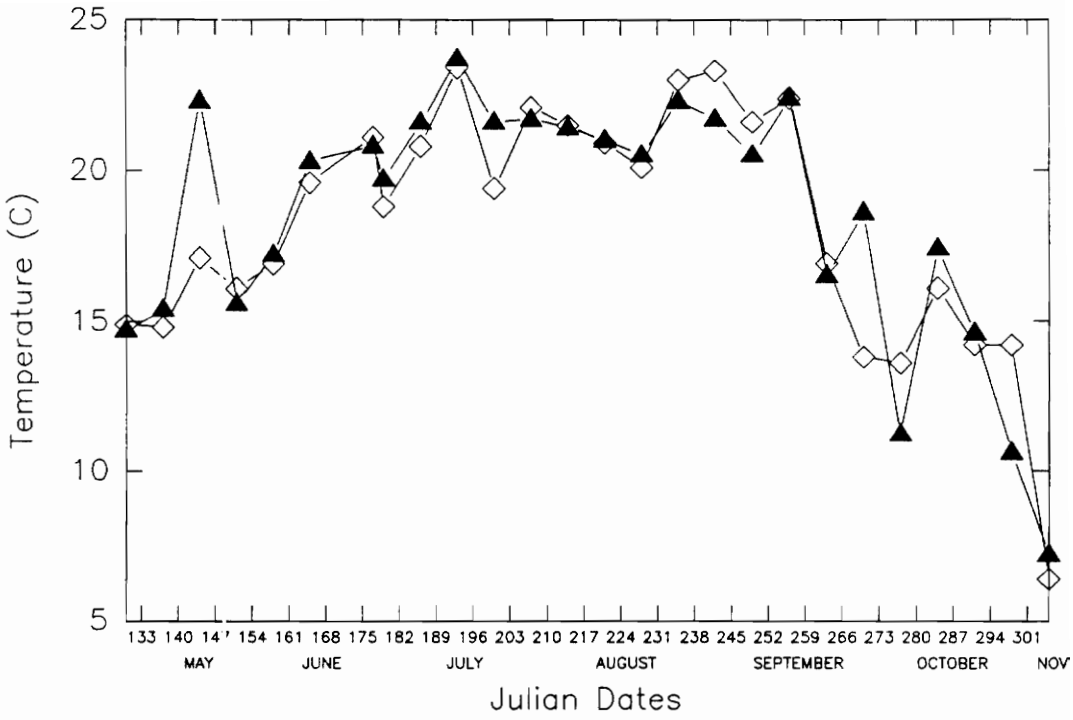

Figure 4.2. Weekly average temperatures in 1990 for Montgomery (filled triangles) and Wythe (open diamonds) Counties, Virginia.

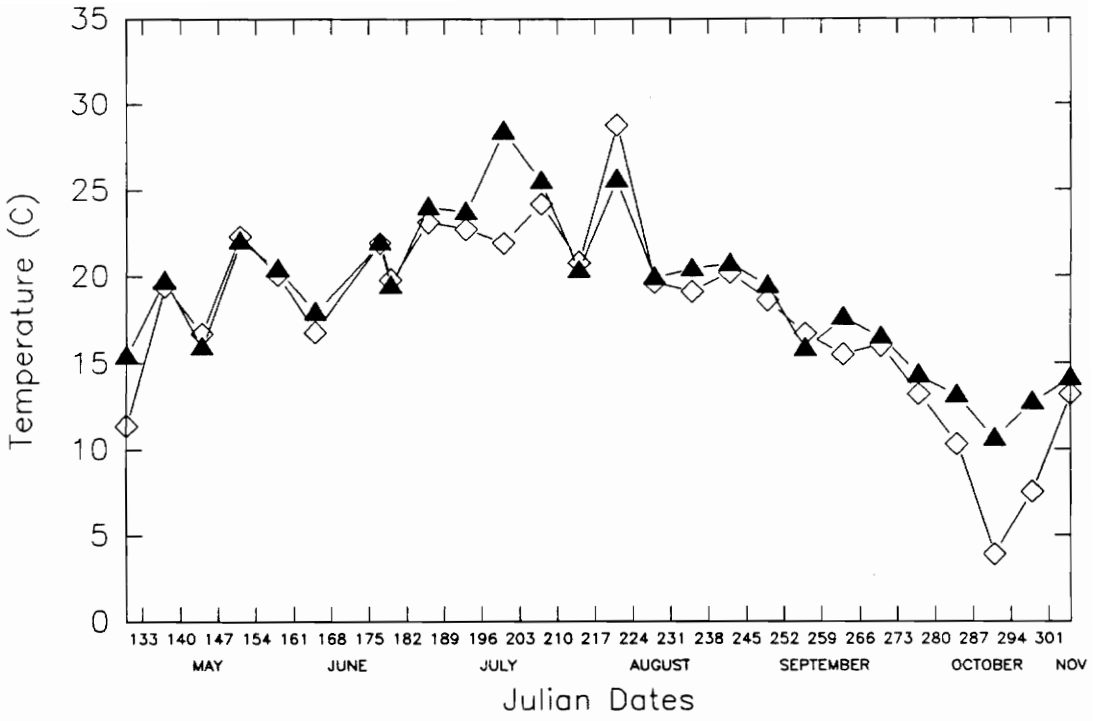

Figure A.3. Weekly average temperatures in 1991 for Montgomery (filled triangles) and Wythe (open diamonds) Counties, Virginia.

Appendix B

Average weekly temperatures for years 1989, 1990, and 1991; specified by location.

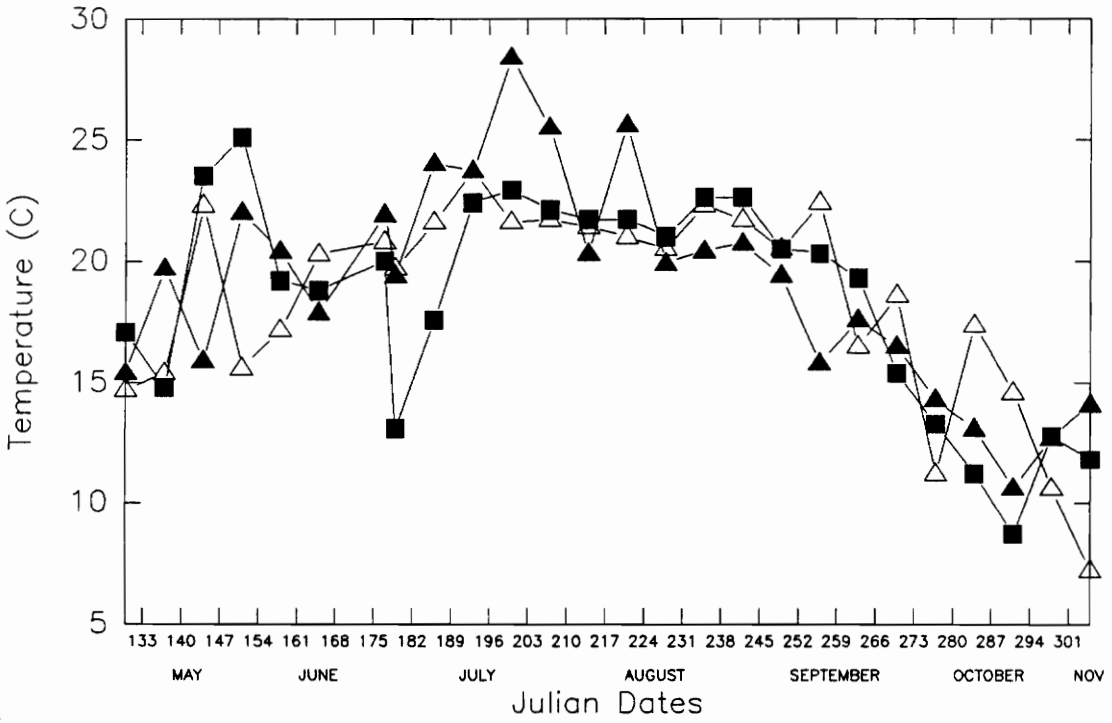

Figure B.1. Weekly average temperature at Montgomery County for 1989 (filled squares), 1990 (open triangles), and 1991 (filled triangles).

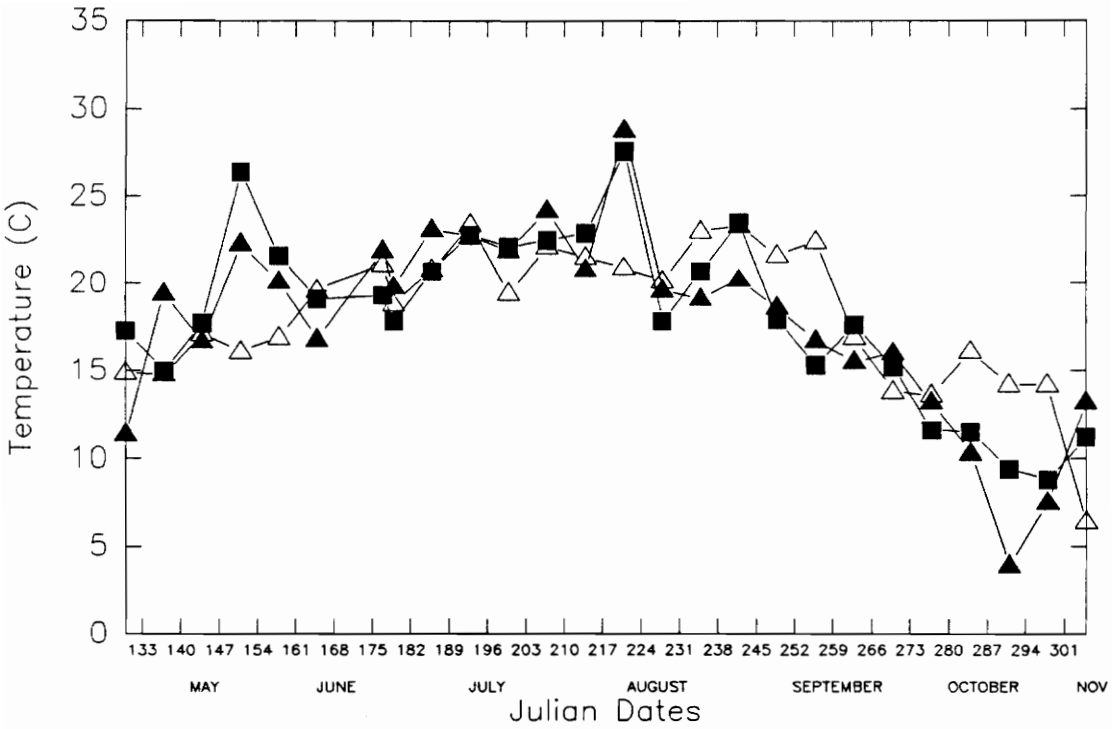

Figure B.2. Weekly average temperatures at Wythe County for 1989 (filled squares), 1990 (open triangles), and 1991 (filled triangles).

Appendix C

Total rainfall at Montgomery and Wythe Counties specified by year.

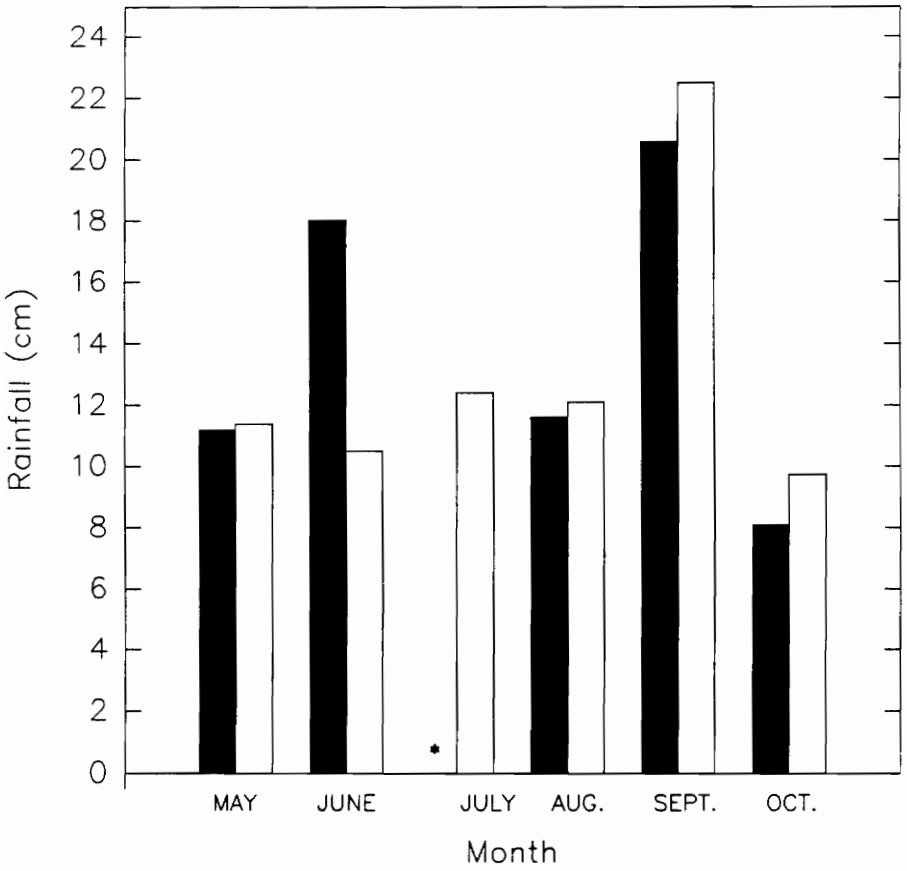

Figure C.1. Average monthly rainfall in 1989 at Montgomery (solid bar) and Wythe (open bar) Counties.
*** denotes data unavailable.**

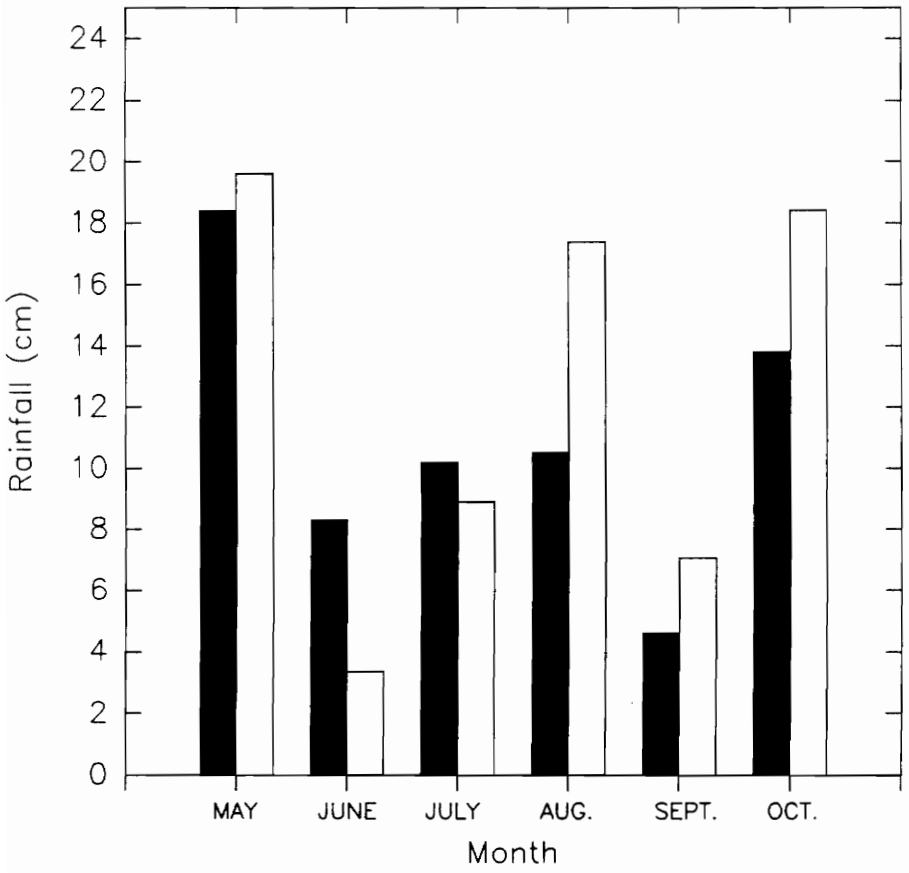

Figure C.2. Average monthly rainfall in 1990 at Montgomery (solid bar) and Wythe (open bar) Counties.

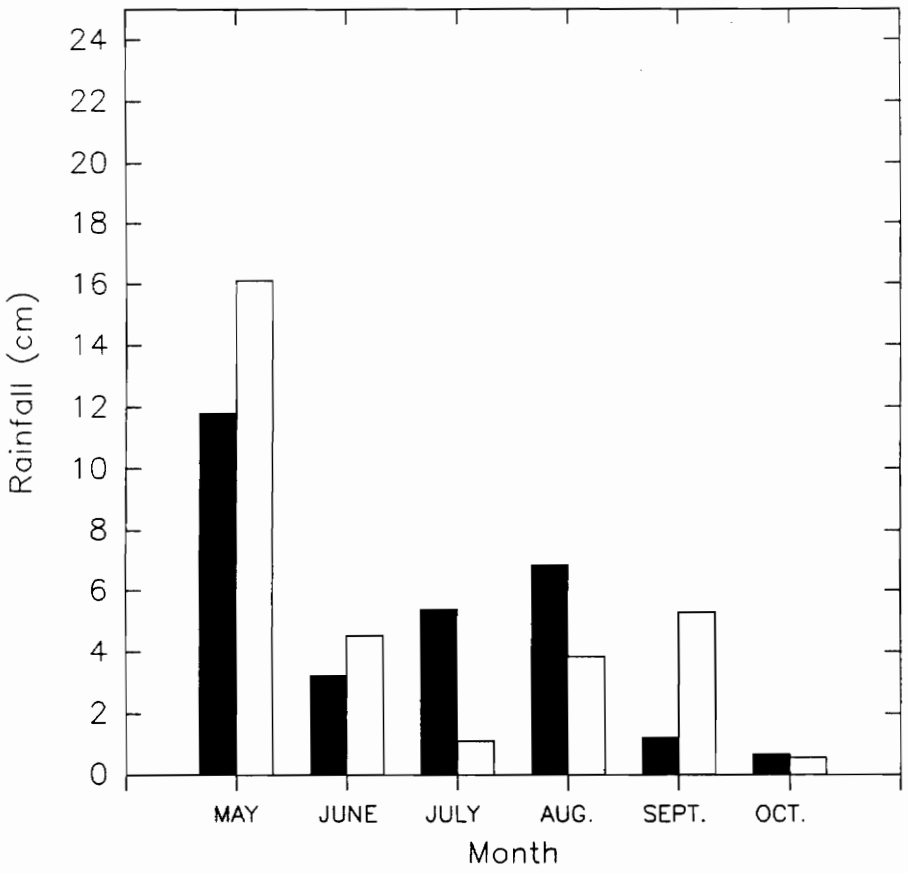

Figure C.3. Average monthly rainfall in 1991 at Montgomery (solid bar) and Wythe (open bar) Counties.

Appendix D

Total rainfall in 1989, 1990, 1991 specified by location.

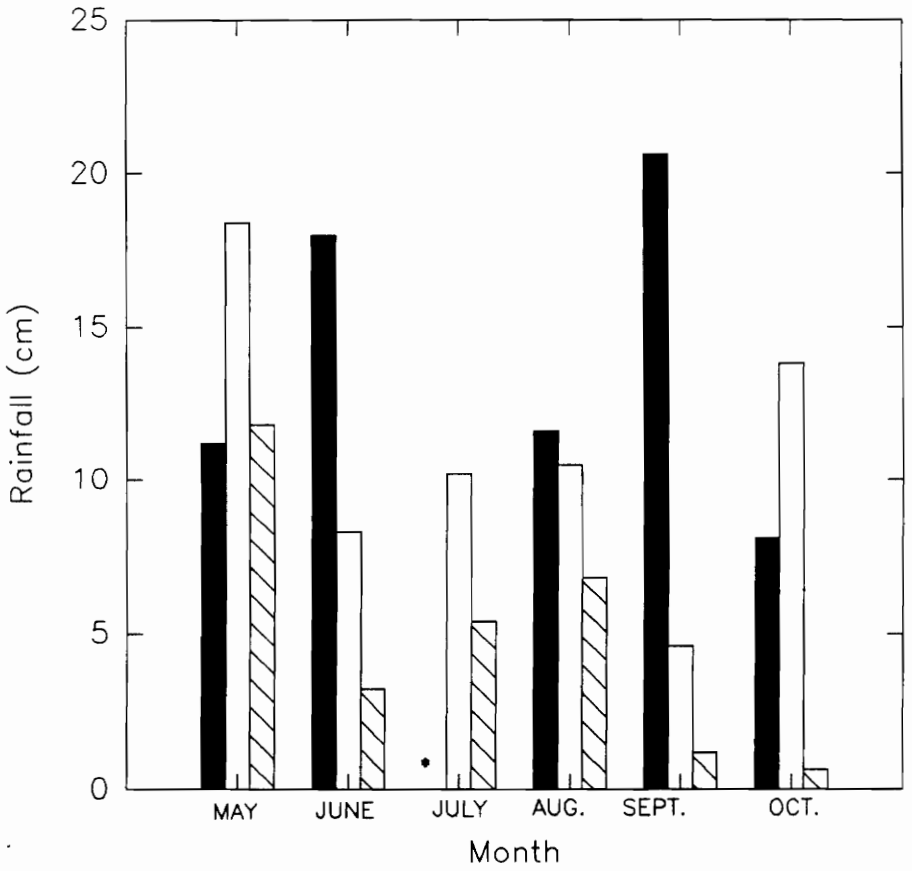

Figure D.1. Average monthly rainfall at Montgomery County, Virginia in 1989 (solid bar), 1990 (open bar), and 1991(lined bar).
*** denotes data unavailable.**

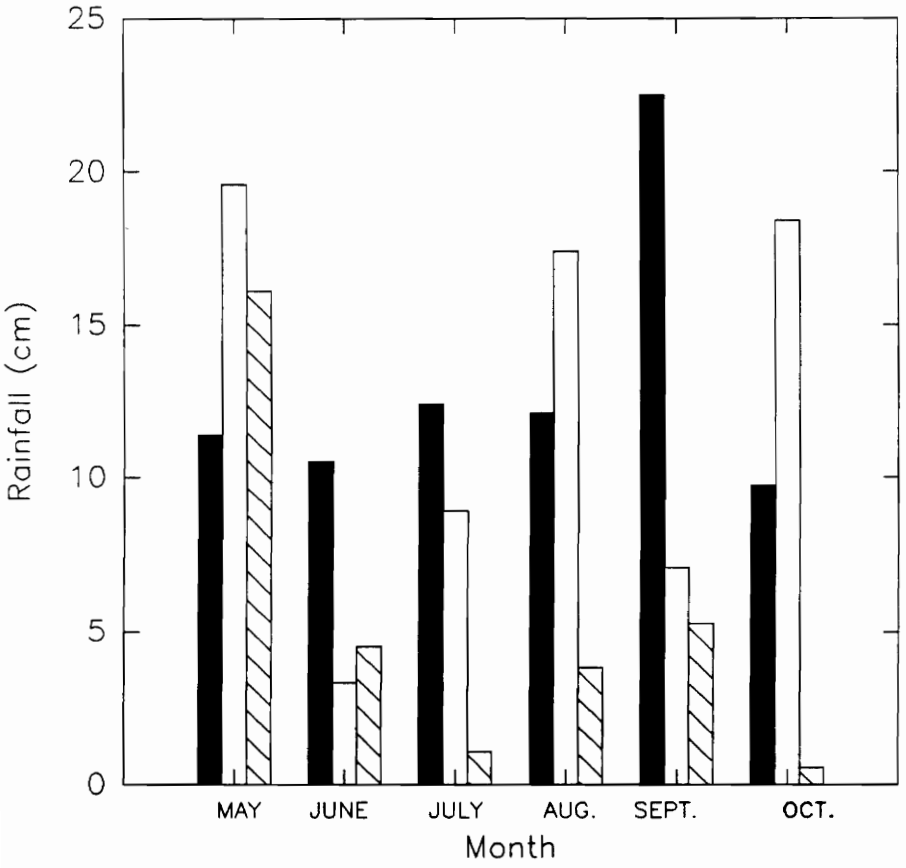

Figure D.2. Average monthly rainfall at Wythe County in 1989 (solid bar), 1990 (open bar), and 1991 (lined bar).

VITA

Michele R. Carter, was born on January 22, 1968 and attended Loudoun Valley High School in Purcellville, VA from 1983-1986. She received her B.S. degree in Biology from Radford University, Radford, VA in 1990. Aside from her academic curriculum, the author performed volunteer research studies on the presence of siderophores in lichen mycobiont isolates from different substrates. During the summers of 1989 and 1990, the author interned for Crop Genetics International, Hanover, MD. Her duties included the performance of technical laboratory and greenhouse practices on two biological projects, *ie.*, INCIDE® and EXTEND®.

In June 1990, she enrolled at Virginia Polytechnic Institute and State University (VPI&SU) serving as a Graduate Research Assistant to the gray leaf spot (GLS) disease of corn project under direction of Dr. Erik L. Stromberg. Duties included the evaluation of commercial corn varieties for resistance to GLS disease, evaluation the efficacy of foliar fungicides to control GLS on corn and blighting assessment, and determination of corn germplasms reaction to cercosporin. The author sought to broaden her training and experiences by competing as a member of the VPI&SU's Weed Science team at the Northeastern Weed Science Societies graduate competition. She received two $1000 dollar scholarships for accomplishments within the department, *ie.*, high GPA, dedication to her research, positions on the Departmental Education Committee and participation in the Graduate Student Organization. She was nominated and accepted as a member of Gamma Sigma Delta Honor Society of Agriculture in 1992. In this same year, she

received a $500 scholarship from the Virginia Ag-Chemical and Soil Fertility Association and won first place in the graduate student paper competition from the American Phytopathological Society, Potomac Division. Upon graduation, the author will be seeking employment as a professional plant pathologist.

The author is the daughter of Richard R. and Deborah W. Carter of Round Hill, VA. Her father is the president of Richard R. Carter, Builder, Inc. and her mother is employed through the corporation as secretary, and treasurer.