Assessment of Losses in Yield and Yield Components of Maize Varieties Due to Grey Leaf Spot

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Abstract

A study was conducted at Bako Agricultural Research Center for three years (1999 – 2001) to quantify the levels of yield loss in maize (*Zea mays* L.) incurred by grey leaf spot (GLS) disease caused by *Cercospora zeae-maydis*. Three commercial varieties with different levels of resistance to GLS, namely, BH-660 (resistant), BH-140 (moderately resistant) and Phb-3253 (susceptible) and three treatments (inoculated, fungicide sprayed and unsprayed control) were used in factorial combination with three replications. Significant differences were observed among varieties for percent infected plants per plot in all the three years and for percent infected leaves per infected plant in 1999 and 2001. The main effect of treatment was also significant for percent infected plants per plot in 1999 and 2000. Variety and treatment effects were significant for disease severity in all years. Variety x treatment interaction was significant for percent upper ear leaf area infected in 1999, and for AUDPC and upper ear leaf area infected in 2000 and 2001. The main effect of year was significant for thousand-kernel weight and ear diameter. Varietal effect was significant for thousand-kernel weight and grain yield, while treatment effect was significant for ear diameter and grain yield. The interactions among year, variety and treatment were non-significant. Mean kernel and grain yield losses ranged from 1.7 to 10.0% and 7.8 to 29.1%, respectively, on different varieties. Grain yield losses in varieties BH-660, BH-140 and Phb-3253 ranged 0–14.9, 13.7–18.3, and 20.8–36.9%, respectively, during the three years. The effect of GLS on ear length and diameter, particularly under natural (unsprayed) condition was not significant. The result indicated that GLS could be severe in some favourable seasons causing significant yield losses even on resistant varieties.

Key words: *Cercospora zeae-maydis,* grey leaf spot, maize, yield loss

Introduction

Owing to its high yield per unit area and adaptability to diverse agro-ecologies, maize (*Zea mays* L.) is among the leading cereal crops selected to achieve food self-sufficiency in Ethiopia (Benti et al. 1993). It is widely produced in most parts of the country and covers about 21% of the nearly 7 million ha of land under cereals cultivation (CSA 2000). Although improved cultivars have been largely included in the national extension package, the current national average yield of maize is only 1800 kg ha^{-1} (CSA 2000), which is far below the world average, 3800 kg ha^{-1} (Dowswell et al. 1996). The low yield is attributed to a combination of several constraints among which diseases play a major role.

Foliar diseases are generally important constraints in tropical maize production (Renfro and Ullstrup 1976). Grey leaf spot (GLS) caused by *Cercospora zeae-maydis* Tehon & Danniels, is now recognized as one of the most significant yield-limiting disease of maize worldwide (Ward and Nowell 1998). Quite recently GLS has become the principal maize disease in Ethiopia. Since 1997, widely distributed epidemics were reported almost every year, especially in warm and humid areas of the country (Dagne et al. 2001). The released varieties currently grown by farmers are severely attacked at localities where the disease intensity is very high.

Losses associated with GLS occur principally when photosynthetic tissues are blighted and prematurely killed by the disease prior to grain filling. The premature death of these tissues seriously restricts accumulation of photosynthate in the developing maize kernel. The dominant sink of the post-anthesis maize plant is the ear. In years of severe blighting, susceptible hybrids develop symptoms that look like frost damage due to necrosis of leaf area (Donahue et al. 1991). Because of reduced photosynthetic areas resulted from blighting, photosynthate is derived from the stalk and roots at a greater-than-normal level causing them to senesce prematurely.

The epidemics of GLS have been frequently reported from different parts of the country in recent years (Dagne et al. 2001). In view of the expansion, seriousness and potential destructiveness of the disease, a number of research activities that could contribute towards the management of GLS have been initiated. However, no quantified information was available on the extent of yield loss it causes on maize production in the country. The current study was, therefore, undertaken to analyze the intensity of grey leaf spot and yield losses incurred by the disease in some popular commercial maize varieties.

Materials and Methods

Experimental Materials and Design

The study was conducted at Bako Agricultural Research Center for three seasons (1999 - 2001). The center is located in western Ethiopia at an altitude of 1650 m. The three commercial hybrids used were: BH-660 (resistant), BH-140 (moderately resistant) and Phb-3253 (susceptible). The treatments were artificial inoculation of the varieties with *C. zeae-maydis*, chemical spraying, and unsprayed control (natural infection). The varieties and treatments were arranged in factorial combination.

The experimental design used was randomised complete block with three replications. Plots were hand-sown during the third week of May in all evaluation years. Each plot consisted of four rows of 5.1 m length spaced at 0.75 m and 0.3 m distance between hills. This gave final plant density of 44,444 plants ha. Phosphate at the rate of 100 kg ha^{-1} was applied at the time of planting while 100 kg ha^{-1} nitrogen was applied in split, one-half at planting and the remaining half 37 days after emergence. Cultural management practices like weeding and slashing were performed as required.

Fungicide Treatment and Inoculation

A systemic fungicide benomyl (Benlate ®) at the rate of 175 g a.i. in 200 l/ha of water was applied using manual knapsack sprayer of 15 l capacity on the sprayed plots. To effectively reduce the blighting, the spray was done 6 times at 7 days interval starting from the time GLS symptom was first observed. Inoculation of the maize varieties with the pathogen was made according to Tembo & Pixley (1999). Infected leaves collected during previous season from infected maize fields were dried and ground into powder and stored in paper bags at a temperature of 4°C. The pulverized leaf was then dusted into the whorls of the plants, where it could be retained long enough to permit spore germination. The inoculation was made at the sixth leaf stage of the crop and was repeated one week later to ensure adequate infection.

Disease assessments

Data were recorded for disease incidence and severity. Disease incidence was recorded one month after mid-silking, when the crop was at grain filling stage as percent infected plants per plot and as percent infected leaves per infected plant. Severity was recorded on plot basis using 1 – 5 scale (i.e., where 1= slight infection and 5 = very heavy infection) as described by Roan et al. (1974) and as percent upper ear leaf area infected. The rating was made 5 times at ten days interval starting when an obvious difference for GLS reaction was observed among the treatments, i.e., at the tenth day after mid-silking. Ten randomly tagged plants were used to record data on percent infected leaves per infected plant and percent upper ear leaf area infected.

Yield and yield component assessments

Grain yield was recorded for the three years at 12.5% moisture while ear length, ear diameter and thousand-kernel weight were taken only in 2000 and 2001. Ear length and diameter were recorded as the average of 10 randomly taken ears from each experimental unit. Thousand-kernel weight was also taken after the moisture was adjusted to 12.5%.

Data analyses

Percent disease incidence and percent upper ear leaf area infected were arcsine transformed before statistical analysis to satisfy the assumption of analysis of variance, since the percentages cover a wide range of values. All disease severity scores were used to calculate area under disease progress curve (AUDPC) for each experimental unit with the formula suggested by Tooley & Grau (1984) as:

$$AUDPC = \sum_{i=1}^{n-1}\left[\left(x_{i+1} + x_i\right)/2\right] * [t_{i+1} - t_i]$$

where x_i = the cumulative disease severity or percent of infected plants at the i^{th} observation, t_i = time (days after planting) at the i^{th} observation and n = total number of observations.

Losses were calculated for ear length, ear diameter, thousand seed weight and grain yield, as the difference between mean yield of fungicide sprayed and artificially inoculated and also mean yield of fungicide sprayed and unsprayed treatments multiplied by hundred and divided by the mean yield of the sprayed treatment. Combined analysis of variance was made over the three years for yield and over the two years for yield components while separate analysis was done for disease parameters collected each year due to heterogeneity of error variances tested using Bartlett's test. Simple correlation analysis was applied to study the relationships among yield, yield components and disease parameters.

Results

Development, incidence and severity of GLS on maize varieties

Grey leaf spot symptoms started to progress rapidly on all varieties at milky stage of the crop, mid August in 1999 and at late August in 2000 and 2001, when obvious experimental unit differences for GLS epidemics became apparent for rating (Figure 1). The disease developed faster in inoculated plots than the fungicide – sprayed or unsprayed plots. Significant differences were observed among varieties for infected plants per plot in all the three years, and for percent infected leaves per infected plant in 1999 and 2001 (Table 1). The main effect of treatment was significant for percent-infected plants per plot in 1999 and 2000. Variety and treatment effects were significant for disease severity in all years.

Variety x treatment interaction was significant only for upper ear leaf area infected in 1999 and for AUDPC and percent upper ear leaf area infected in 2000 and 2001. The values of all the disease parameters were generally higher for the variety Phb-3253 while lower values were observed on BH-660 in all years (tables 2–5). The variety BH-140 generally showed intermediate infection levels for the disease parameters although in some cases it exhibited lower or equal levels to BH-660 when sprayed (tables 3–5). Plots inoculated with *C. zeae-maydis* showed higher crop injury than the sprayed and unsprayed treatments for all varieties in each year. The sprayed treatment showed lower disease intensity in 1999 whereas the unsprayed treatment exhibited lower values for most disease parameters in 2001 (tables 2, 3 and 5).

Effect of GLS on grain yield and yield components of maize

The combined analyses of variances showed significant differences among years for thousand-kernel weight and ear diameter (Table 6). The main effect of variety was significant for thousand-kernel weight and grain yield while the main effect of treatment was significant for ear diameter and grain yield. All the interaction effects were non-significant for grain yield and all yield components evaluated.

The effect of GLS on ear length and diameter was very low as compared to its effect on kernel weight and grain yield. There were no losses in ear length and diameter of BH-660 and were very small in the other varieties even after inoculation (Table 7). Losses in kernel weight were 1.7, 7.9 and 10.0 (data not presented) percent in BH-660, BH-140 and Phb-3243, respectively, when inoculated. No kernel weight loss was recorded under natural infection condition (unsprayed). Losses in grain yield ranged from 8 to 29 for the different varieties when inoculated. Similar losses were obtained for BH-660 when inoculated and under natural infection condition, whereas considerably higher losses were recorded under inoculation than natural condition in the other varieties. The overall highest grain yield (10.30 t ha^{-1}) was obtained from BH-660 under fungicidal disease control and the lowest (5.64 t ha^{-1}) was from Phb-3253 in artificially inoculated plots. All varieties showed lower yield, but with different magnitude, when artificially inoculated with the pathogen, whereas higher yield was obtained from plots sprayed with fungicide. Yield losses ranged from none for BH-660 in 2001 to 36.9% for Phb-3253 in 2000 over the three years (Figure 2).

The associations among yield, yield components and disease parameters are presented in Table 8. Positive and highly significant correlations were observed among all disease parameters. In most cases, the associations between yield and yield components, and disease parameters were negative and significant. The associations of grain yield loss in artificially inoculated plots with AUDPC and percent of upper leaf area infected were highly significant with positive correlation coefficients of 0.80 and 0.82 (n = 9), respectively (data not presented). However, other yield losses exhibited non-significant correlation with disease parameters.

Table 1. Analysis of variance of grey leaf spot disease parameters for the 1999 – 2001 cropping seasons at Bako

Year	Source of variation	Degree of freedom	Mean squares			
			Incidence		Severity	
			Infected plants per plot (%)	Infected leaves per plant (%)	Upper ear leaf area infected (%)	AUDPC[a]
1999	Variety (v)	2	4723.7**	3223.8**	3491.9**	9594.7**
	Treatment (T)	2	1473.9*	749.4	1723.1**	3501.6**
	V x T	4	601.5	511.8	297.6**	196.8
	Error	16	350.6	233.3	48.5	212.7
2000	Variety (V)	2	831.9**	22.8	2768.7**	5618.3**
	Treatment (T)	2	237.0*	34.0	904.4**	3462.0**
	V x T	4	120.1	10.7	113.1**	339.1**
	Error	16	58.8	10.9	20.3	39.3
2001	Variety (V)	2	3136.6**	376.9**	1469.1**	4034.0**
	Treatment (T)	2	171.7	60.8	385.8**	1034.0**
	V x T	4	97.1	37.4	188.8*	220.1**
	Error	16	53.0	38.8	45.6	33.8

* & ** = significant at 0.05 and 0.01 probability level, respectively; [a]Disease severity (1-5 scale) as expressed by area under the disease progress curve.

Table 2. Effect of maize hybrids and fungicide treatment on grey leaf spot disease incidence for 1999 – 2001 cropping seasons at Bako

Variety	**Incidence**					
	Infected plants per plot (%)			Infected leaves per plant (%)		
Treatment	1999	2000	2001	1999	2000	2001
Variety						
BH-660	52.8	86.8	75.8	93.5	98.9	93.5
BH-140	49.4	89.2	65.2	96.1	99.6	96.1
Phb-3253	100.0	99.7	100.0	99.8	100.0	99.8
Treatment						
Inoculated	86.1	96.1	84.1	89.7	99.9	97.8
Unsprayed	56.7	87.5	72.9	79.4	99.9	94.0
Sprayed	59.4	92.1	84.0	65.9	98.7	97.6
Mean	67.41	91.88	80.33	96.47	99.49	96.47
CV(%)	27.78	8.35	9.06	15.83	3.32	6.46

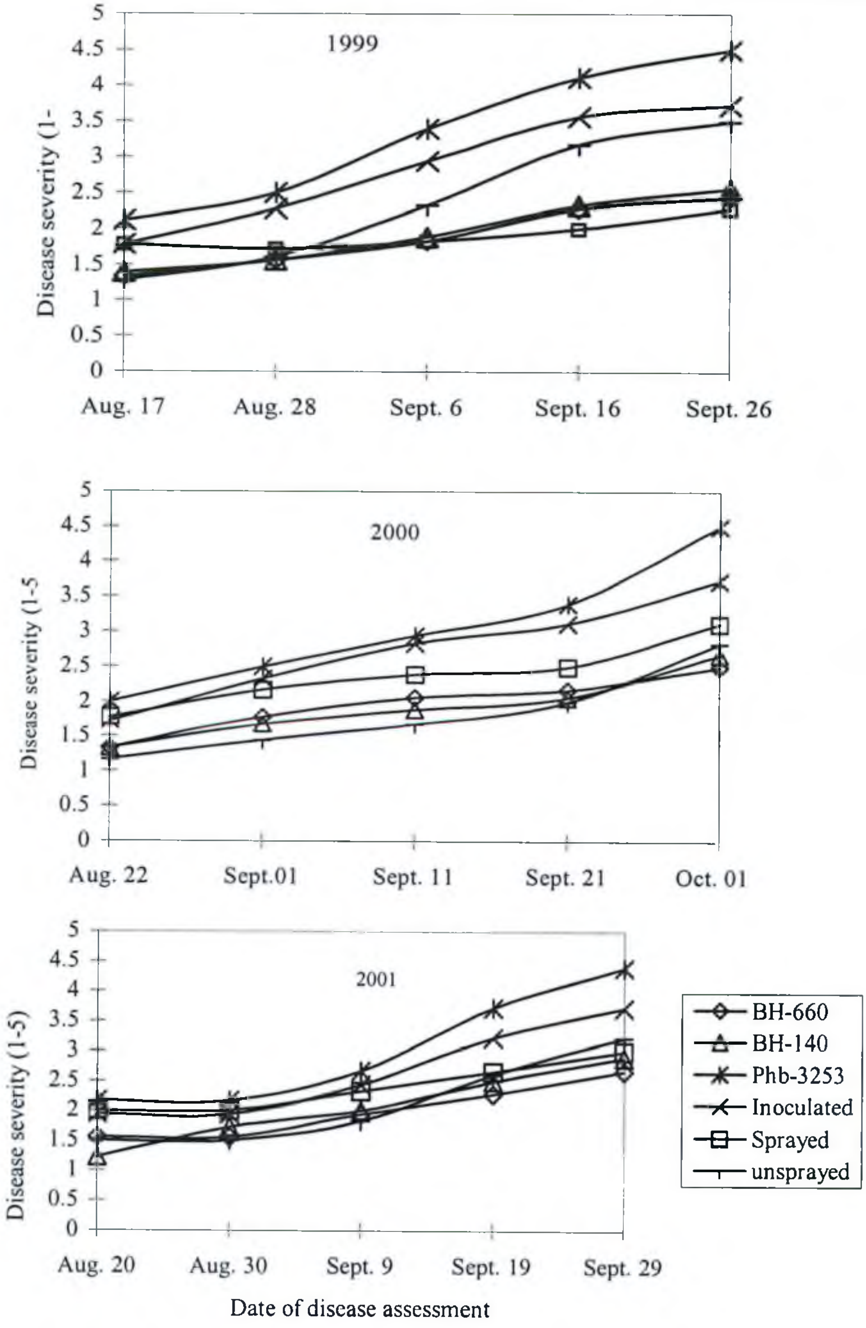

Figure 1. Disease severity progress curves of grey leaf spot under inoculated, unsprayed and fungicide sprayed treatments on three maize varieties at Bako during 1999, 2000 and 2001 cropping seasons

Disease severity score was based on 1–5 rating scale, where 1 = slight infection and 5= very heavy infection.

Table 3. Mean grey leaf spot disease severity of three maize hybrids under inoculated, unsprayed and sprayed treatments at Bako in 1999

Variety	Disease severity					
	Upper ear leaf area infected (%)			AUDPC[a]		
	Inoculated	Unsprayed	Sprayed	Inoculated	Unsprayed	Sprayed
BH-660	21.8	11.2	9.0	95.8	68.3	62.5
BH-140	31.8	27.4	2.6	90.8	82.5	59.2
Phb-3253	95.0	78.7	26.0	159.2	134.2	105.8
Mean	49.53	39.10	12.53	115.27	95.00	75.83

[a]Disease severity (1–5 scale) as expressed by area under the disease progress curve; CV (%) = 30.88 for upper ear leaf area infected (%) and 15.29 for AUDPC

Table 4. Mean grey leaf spot disease severity of three maize hybrids under inoculated, unsprayed and sprayed treatments at Bako in 2000

Variety	Disease severity					
	Upper ear leaf area infected (%)			AUDPC[a]		
	Inoculated	Unsprayed	Sprayed	Inoculated	Unsprayed	Sprayed
BH-660	53.5	32.8	20.5	90.8	60.0	86.7
BH-140	61.7	65.5	20.8	90.0	65.0	73.3
Phb-3253	95.0	86.7	80.0	149.2	88.3	125.0
Mean	70.07	61.67	40.43	110.00	71.10	95.00

[a]Disease severity (1–5 scale) as expressed by area under the disease progress curve; CV (%)= 12.16 for upper ear leaf area infected (%) and 6.81 for AUDPC.

Table 5. Mean grey leaf spot disease severity of three maize hybrids under inoculated, unsprayed and sprayed treatments at Bako in 2001.

Variety	Disease severity					
	Upper ear leaf area infected (%)			AUDPC[a]		
	Inoculated	Unsprayed	Sprayed	Inoculated	Unsprayed	Sprayed
BH-660	23.3	10.3	23.3	85.0	71.7	80.0
BH-140	17.7	11.3	24.0	89.2	79.2	87.5
Phb-3253	79.3	40.3	39.0	139.2	98.3	117.5
Mean	40.10	20.63	28.77	104.47	83.07	95.00

[a]Disease severity (1–5 scale) as expressed by area under the disease progress curve; CV (%)= 35.43 for upper ear leaf area infected (%) and 6.17 for AUDPC.

Table 6. Mean squares from combined analysis of maize grain yield and some yield components at Bako during 1999, 2000 and 2001 cropping seasons

Source of variation	Degrees of freedom[c]	Mean squares			
		Grain yield	Thousand kernel weight	Ear length	Ear diameter
Year (Y)[b]	2(1)	8.3	66685.9**	1.53	0.13**
Replication within year (R/Y)	6(4)	2.8	992.6	4.10**	0.02
Variety (V)	2(2)	54.2**	6214.8*	21.80	0.24
Y x V	4(2)	1.42	255.7	12.30	0.03
Treatment (T)	2(2)	15.9*	3763.9	2.22	0.13**
Y x T	4(2)	1.5	618.3	0.48	0.001
V x T	4(4)	1.8	287.9	0.39	0.02
Y x V x T	8(4)	1.09	1405.5	2.09	0.03
Pooled error	48(32)	1.97	670.2	0.93	0.02

* and ** = significant at 0.05 and 0.01 probability level, respectively, using R/Y as an error term for Y, Y x V for V, Y x T for T, Y x V x T for Y x V, Y x T and V x T, pooled error for R/Y and Y x V x T; [b]Year is considered as a random variable;

* [c]Degrees of freedom in parenthesis are for mean squares for thousand-kernel weight, ear length and ear diameter;

* Grain yield was combined over 3 years while the yield components were combined over 2000 and 2001 cropping seasons

Table 7. Effect of grey leaf spot on mean grain yield of three maize varieties at Bako. Means were calculated over all data from the 1999, 2000 and 2001 cropping seasons

Variety	Treatment	Ear length (cm)	Ear diameter (cm)	Thousand kernel weight (g)	Grain yield	
					t/ha	% loss
BH-660	Inoculated	19.5	4.4	325.0	9.5	8
	Unsprayed	19.9	4.6	350.1	9.4	9
	Sprayed	19.5	4.4	331.6	10.3	-
BH-140	Inoculated	17.6	4.5	285.1	7.5	17
	Unsprayed	18.3	4.6	313.6	8.2	9
	Sprayed	18.4	4.7	309.5	9.0	
Phb-3253	Inoculated	17.3	4.6	283.8	5.6	29
	Unsprayed	18.2	4.8	314.7	7.1	10
	Sprayed	17.4	4.7	315.4	8.0	-
	Mean	18.50	4.60	314.42	8.29	
	CV.%*	5.21	3.10	8.23	16.90	

* CV = coefficient of variation.

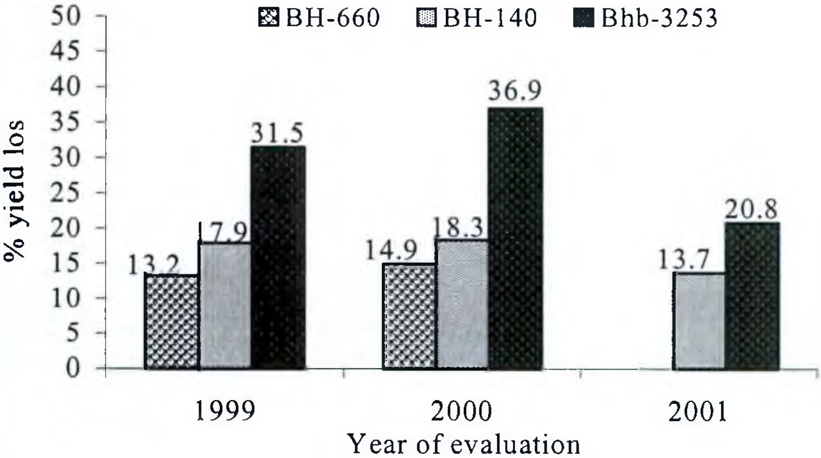

Figure 2. Grain yield loss of maize varieties due to grey leaf spot at Bako during 1999, 2000 and 2001 cropping seasons
Loss was not observed in BH-660 in 2001

Table 8. Coefficients of correlation among yield, yield components and disease parameters of three maize varieties at Bako during the 1999 - 2001 cropping seasons

	AUDPC	%IPPP	%UELAI	%ILPP	EL	ED	TKW
AUDPC[1]	-						
% IPPP	0.58**	-					
% UELAI	0.74**	0.63**	-				
% ILPP	0.39**	0.70**	0.45**	-			
EL	-0.45**	-0.20	-0.46**	-0.21	-		
ED	0.13	0.03	0.06	0.15	-0.11	-	
TSW	-0.24	-0.49**	-0.56**	-0.52**	0.38**	0.28*	-
Grain yield	-0.62**	-0.24*	-0.63**	-0.02	0.63	0.02	0.51**

* and ** = significant at 0.05 and 0.01 level of significance;
[1]AUDPC = Area under disease progress curve calculated from disease severity score, % IPPP = percent infected plants per plot, % UELAI= percent upper ear leaf area infected, % ILPP = percent infected leaves per plant, EL = ear length, ED = ear diameter percent, and TKW= thousand kernel weight

Discussion

The hybrid varieties used in this experiment were found to have different levels of reactions to GLS (tables 2–5). As it was expected, BH-660. consistently showed lower levels of leaf blighting while the susceptible hybrid, Phb-3253, was highly damaged by the disease in each year. Epidemiologically, infested crop residue is the most important source of *C. zeae-maydis* inoculum (de Nazareno et al. 1993). Similarly, the disease pressure has been significantly increased in the current experiment by the artificial inoculation of the plants using infected leaf tissue from the previous season. The crop was not well protected by fungicide treatment in 2000 and 2001 (tables 2, 4 and 5). This might have been due to the late application of the fungicide after GLS has made the damage to many leaves of most plants. The timing of application of fungicide is, thus, critical, and the most effective time to commence treatment is earlier than that. This occurs when the lesions are visible only on the basal five-leaves of the plant (Ward et al. 1997).

The significant variety x treatment interaction observed for GLS indicated that the hybrids reacted differently to fungicide treatment. The main effect of year was significant for thousand-kernel weight and ear diameter, implying the presence of significant variation among years for hybrid performance in these traits. Considerable differences were also observed among the varieties for thousand-kernel weight and grain yield. The higher yield obtained from BH-660 in all treatments indicated the genetic potential of the hybrid for high yield and disease resistance. The lower yield loss recorded on BH-660 further confirmed the resistance of the hybrid. The most yield limiting grey leaf spot epidemic was observed in artificially inoculated plots. Similar result was reported by Carter and Stromberg (1992). Ward et al. (1997) reported that fungicides are used to effectively and economically manage grey leaf spot epidemics in a limited number of commercial grain maize production in the United States, as well as in seed and feed maize productions in South Africa. The results of the current experiment in 1999 confirmed this finding. In some cases, however, the disease intensity was higher under natural infection, indicating that the presence of pathogen is not the only factor for disease development. According to Ward et al. (1998), despite the inoculum load, the GLS disease develops fast if the environment is conducive for the fungus.

The higher yield loss measured in artificially inoculated plots indicated the increase in the absolute rate of disease development as the amount of inoculum increases. Reduction in grain yield and in some yield components due to increased disease pressure was associated principally to increased blighting and premature death of photosynthetic tissues prior to grain filling. Yield is a function of photosynthesis and is related to a healthy leaf area and its duration after flowering (Eik and Hanway 1966). The premature death of the tissues seriously restricts the accumulation of photosynthate in the developing corn kernels (Donahue et al. 1991). The level of yield loss observed in this experiment signifies that GLS was of greater importance under inoculum concentration. The higher yield loss observed in 2000 might have also been attributed to the high rainfall and relative humidity early in the growing seasons (data not presented). The appearance of lesions earlier in the growing season allows host and pathogen populations to interact over a longer period of time (Nutter and Stromberg 1999). In years of severe epidemics, the disease may cause significantly higher yield loss than what was observed in this experiment. In hybrid evaluation trial conducted in South Africa, yield loss of up to 50% occurred in hybrids with moderate resistance and 65% in susceptible hybrids (Ward et al. 1997). After three years of treatment with different fungicides and application intervals, Carter and Stromberg(1992) observed yield increases of 31 to 66% depending on the fungicide and treatment regime.

The close association among disease parameters indicated that all the methods of disease assessment used in the present experiment could express the relative damage level of the varieties under different disease pressure of the GLS. However, severity score at different stages of the crop is the method of choice for GLS evaluation. This method is easy, speedy and economical, and also used to calculate AUDPC over many ratings made throughout the season. Data on disease incidence seem to be less important as it indicates prevalence, but not intensity of GLS.

The study clearly indicated that use of resistant genotypes in disease-prone areas is the major component of disease management practices. Farmers have to use varieties that are less susceptible to GLS, and also with high yield potential and stability. Varietal resistance, however, has to be complemented with other appropriate measures such as residue management of the previous crop as components of integrated pest management approaches.

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