Reaction of Improved Maize (*Zea mays* L.) Varieties to Grey Leaf Spot (*Cercospora zeae-maydis*) in South and Southwest Ethiopia

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Abstract: Grey leaf spot (GLS) is one of the most important diseases that constrain maize production and productivity in maize-growing areas of Ethiopia where a warm humid environmental condition prevails. Thus, this study was conducted to evaluate the reaction of 12 maize varieties to maize grey leaf spot under field conditions in Hawassa and Jimma, south and southwest Ethiopia, in 2014 and 2015 cropping seasons. The treatments consisted of twelve maize varieties. The experiment was laid out as a randomized complete block design (RCBD) and replicated three times per treatment. Disease severity was assessed as the proportion of leaf area affected by the disease on 10 randomly tagged plants in the middle two rows. The area under disease progress curve (AUDPC) and disease progress rates were estimated from the percent severity index (PSI). Similarly, grain yield was determined after harvest and converted into yield per hectare. The results revealed that the final disease severity varied from 37.33 to 84.83 PSI and 39.5 to 81.83 PSI at Jimma; 35.67 to 78.12 PSI and 35.67 to 78.12 PSI at Hawassa in 2014 and 2015 main cropping seasons, respectively. AUDPC varied from 1426.67 to 3281.67%-days in Jimma and from 1476.67 to 3225%-days in Hawassa in 2014 main cropping season; 1176.17 to 3031.67%-days in Jimma and 1226.67 to 2975%-days in Hawassa in 2015 main cropping season. The varieties Gibe-2, BH-543 and Local-K exhibited high disease severity, high AUDPC and high infection rate and were categorized as highly susceptible maize varieties. The results also indicated that BH-660 and BH-670 were considered resistant to grey leaf spot across the two locations and had low disease severity as well as low AUDPC values. It is concluded that under field conditions, different maize varieties responded differently to grey leaf spot and the disease severity was strongly affected by the use of different resistance levels of maize varieties and difference in the environmental conditions. It is therefore, promising to use the two maize varieties, such as BH-660 and BH-670, were considered as resistant under field conditions and that are recommended to be used by farmers in the study areas and elsewhere with similar agro-ecologies in Ethiopia.

Keywords: AUDPC, disease progress rate, disease severity index, grey leaf spot, grain yield, *Zea mays*.

1. Introduction

Maize (*Zea mays* L.) is one of the most important food crops world-wide. It is the principal component of human diet and feed constituent for domestic animals. It ranks third in production worldwide following wheat and rice (FAOSTAT, 2012), and is grown in most parts of the world over a wide range of environmental conditions, with altitudinal ranges from 0 to 3000 m above sea level (Dowswell *et al*., 1996). In Africa, maize is grown by small and medium-scale farmers who cultivate 10 hectares or less (Devries and Toenniessen, 2001). In the region, the use of agricultural input is extremely low resulting in poor average yield of 1.3 tons per hectare (Bänziger and Diallo, 2004). Regardless of poor or low productivity, maize production area is rapidly increasing in Sub-Saharan Africa, including the marginal areas (FAOSTAT, 2012).

In Ethiopia, maize is one of the most important cereal crops grown. Among all cereals, maize ranks second to tef (*Eragrostis tef*) in area coverage but first in productivity and total production (CSA, 2014). Maize is currently produced by more farmers than any other crops.

At the national level, there are about 8.8 million maize cropping smallholder farmers in Ethiopia (CSA, 2012). In view of its importance, extensive adaptation, total production and productivity, maize is considered as one of the most priority food security crops in Ethiopia (CSA, 2012). However, maize yields have remained low due to several biotic, abiotic and socio-economic constraints (Mosisa *et al*., 2012). The predominant biotic constraints of maize production in Ethiopia are (diseases, weeds, insect insects and other arthropod pests), abiotic (drought and nutrient deficiencies) and socio-economic (market price fluctuation, and unavailability of inputs) constraints that limit maize productivity in Ethiopia (Tesfa *et al*., 2004; Mosisa *et al*., 2012). Among the biotic factors, diseases are the principal threats limiting maize production and productivity. Foliar diseases, including grey leaf spot (*Cercospora zeae-maydis* Tehon and

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Daniels), *Turcicum* leaf blight (*Exserohilum turcicum* Pass Leonard and Suggs) and common leaf rust (*Puccinia sorghi* Schr.) (Tewabech *et al.*, 2012) are the most important infectious diseases of maize in the country. Compared to other leaf diseases, grey leaf spot is the most widely distributed and has high economic importance (Tewabech *et al.*, 2012).

Grey leaf spot is particularly important in Africa because maize is the main staple food crop for millions of people in the rural areas (Ward *et al.,* 1999). This foliar disease has the potential to threaten food security in many countries (Ward *et al.,* 1999). The disease causes necrotic lesions that may coalesce and cause extensive blighting of leaves, thereby reducing the photosynthetic area of maize plants. Consequently, it may result in poor grain filling, which leads to low maize yields (Kinyua *et al.*, 2010) that impacts the yield. According to Allison and Watson (1996), the upper eight or nine leaves of the plant contribute 75 to 90% of the photosynthate for grain filling. The premature death of these tissues due to infection seriously restricts accumulation of photosynthates in the developing kernel. 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 resulting from blighting, photosynthate is derived from the stalk and roots at a greater than normal level causing them to senesce prematurely.

Among the major disease constraints on maize production in Ethiopia, diseases such as grey leaf spot result in high yield losses due to high grey leaf spot incidence and severity in the farmers' fields. Cultural methods and use of fungicides have been used for grey leaf spot management (Ward *et al.*, 1997), but have not been effective because fungicide application is costly and not practical in most operations for the resource-poor farmers and also unpredictable weather and the environmental side effects (Danson *et al.*, 2008). Availability and adoption of resistant maize hybrids would provide a cost-effective means of controlling grey leaf spot (Ininda *et al.*, 2007). However, little empirical information is available regarding the reaction of several maize varieties to the disease. Therefore, the objective of this study was to evaluate the reaction of improved maize varieties to maize grey leaf spot under field conditions.

2. Materials and Methods

2.1. Description of the Experimental Sites

The grey leaf spot evaluation of field trials was made on 12 maize varieties planted at two locations in 2014 and 2015 main cropping seasons. Field experiments were conducted at two different locations of south and southwest Ethiopia, namely Eladale (Experimental field of Jimma University College of Agriculture and Veterinary Medicine) and Hawassa Agricultural Research Center (HARC) in the two consecutive main rainy seasons. The field experiment was conducted on clay soil at Eladale and clay loam soil at Hawassa Agricultural Research Center (HARC) during the main cropping seasons. Jimma (Eladale-JUCAVM field) is located at 7°42'N and 36°48'E with an altitude of 1813 m.a.s.l. in southwest Jimma Zone at around 8 km away from Jimma University College of Agriculture and Veterinary Medicine (JUCAVM). Jimma (Eladale-JUCAVM field) is also characterized by extended higher precipitation (estimated to exceed 1616 mm per annum) and many rainy days than Hawassa during the cropping periods with mean daily temperatures ranging between 12.4 and 28.4°C (Mulugeta *et al.*, 2011), while Hawassa Agricultural Research Center (HARC) is located at 7°4'N and 38°31'E with an altitude of 1700 m.a.s.l. in Southern Nations, Nationalities and Peoples' Region (SNNPR). The mean annual rainfall for the location is 1072 mm during the main cropping season with mean minimum and maximum temperatures of 14.1 and 26.3°C, respectively (Waga, 2011).

2.2. Land Preparation, Experimental Materials, Treatments and Design:

2.2.1. Land Preparation

The land was prepared by plowing two times in each cropping season. Recommended fertilizers DAP and Urea at a rate of 100 kg ha^{-1} each was applied. It was performed by applying DAP at the time of planting and Urea was applied when the maize plants reached at knee height. Cultivation and weed management were carried out three times after planting at the two locations of Jimma and Hawassa in 2014 and 2015 main cropping seasons.

2.2.2. Experimental Materials

Eleven released maize varieties (BH-660, BH-540, BH-140, BH-543, BHQPY-545*, BH-670, BH-661, Gibe-2, Morka, Kuleni, and Gibe-1) with different levels of resistance and one local check variety, totally 12 maize varieties, were evaluated for their reaction to grey leaf spot at two locations (Jimma and Hawassa) in 2014 and 2015 main cropping seasons (Table 1).

2.2.3. Treatments and Experimental Design

The treatments consisted of twelve maize varieties tested at two locations of Jimma and Hawassa in 2014 and 2015 cropping seasons. The experiments were conducted under naturally-infected fields. Each plot size was 3 m x 3 m (= 9 m^2) consisting of four rows with 30 cm intra-row and 75 cm inter-row spacing's. The whole plots were bordered on four sides with three infector rows of the susceptible variety, Local-K. The space between plots was 1 m and there was 1.5 m space between blocks. The total area of the land used for this experiment was 16 m x 51 m (816 m^2). Seeds were planted in rows with two seeds per hill and also seedlings were thinned into one plant per hill four weeks after emergence. In each plot 40 plants were grown and each row had 10 maize plants. The treatments were arranged in a randomized complete block design (RCBD) with three replications. Sowing was done on April 19 and April 28 in 2014 and 2015 cropping seasons at Jimma and April 27 and May 18 in 2014 and 2015 cropping seasons at Hawassa, respectively.

Table 1. Description of maize varieties with their agro-ecological adaptation and agronomic characters used in this study at Jimma and Hawassa, south and southwest Ethiopia.

Varieties	Year of Release	Altitude (m)	Rainfall (mm)	Plant height (cm)	Reaction to GLS	Released by
BH-660	1993	1600-2200	1000-1500	255-290	T	BARC
BH-540	1995	1000-2000	1000-1200	240-260	MT	BARC
BH-140	1988	1000-1700	1000-1200	240-255	MT	BARC
BH-543	2005	1000-2000	1000-1200	250-270	MT	BARC
BHQPY-545*	2008	1000-1800	1000-1200	250-260	T	BARC
BH-670	2001	1700-2400	1000-1500	260-295	T	BARC
BH-661	-	-	-	-	-	BARC
Gibe-2	2011	-	-	-	-	BARC
Morka	2008	1600-1800	1200-2000	270-300	T	JARC
Kuleni	1995	1700-2200	1000-1200	240-265	T	BARC
Gibe-1	2000	1000-1700	1000-1200	240-260	MT	BARC
Local-K	-	-	-	-	S	-

Note: T = Tolerant; MT = moderately tolerant; S = susceptible; where: BARC = Bako Agricultural Research Center, JARC = Jimma Agricultural Research Center. Source: Mandefro et al. (2009).

2.3. Disease Incidence and Severity Assessments

Field disease assessment at each location was assessed 6 times throughout the growing season from onset of the disease until the maize reached the dent stage (Ringer and Grybauskas, 1995). Ten randomly taken plants in the two central rows were tagged and used for successive disease assessments.

Disease incidence (%): The progress of percentage incidence of disease in maize was quantified in staggered plant at 10 days intervals starting from onset of disease to dent stages or ratio of infected leaves to the total number of leaves on a particular plant and expressed as a percentage. The percentages of disease incidence were calculated by using the following formula suggested by Cooke *et al.* (2006).

$$\text{Incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total no of plants assessed}} \times 100 \qquad (1)$$

Disease severity (%): Disease severity was rated using 1-5 scales described by Maroof *et al.* (1993). The scores were described as 1 = no symptoms; 2 = moderate lesion development below the leaf subtending the ear; 3 = heavy lesion development on and below the leaf subtending the ear with a few lesions above it; 4 = severe lesion development on all but the uppermost leaves, which may have a few lesions; and 5 = all leaves dead. Rating started when obvious genotypic differences for GLS reaction became apparent and continued until leaves senesced. Disease severity scores were converted into percentage severity index (PSI) for analysis using the following formula suggested by Wheeler (1969) as given below.

$$\text{PSI} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total No. of rated} \times \text{Max. disease score on scale}} \qquad (2)$$

2.4. Area under Disease Progress Curve (AUDPC)

The area under disease progress curve, which consists of proportions of diseased plants, was calculated from disease severity recorded at ten days interval starting from the onset of disease 6 times in each location throughout the growing period and converted to percent severity index (PSI). To ensure consistent disease evaluation in the field, the area under disease progress curve was calculated. This curve was developed from 10 days disease severity reading in different locations. By constructing a curve, symptom development and disease severities were compared over years and locations. The area under disease progress curve (AUDPC) is used to quantify suppressing of the beginning of the epidemic and the time until the grey leaf spot reached peak. Grey leaf spot (GLS) for the whole plant was converted to AUDPC to compare relative level of resistance and susceptible varieties. Area under disease progress curve (AUDPC) was computed from severity data using the formula suggested by Campbell and Madden (1990) as:

$$\text{AUDPC} = \sum_{i=1}^{n} [0.5\,(x_i + x_{i+1})]\,[t_{i+1} - t_i] \qquad (3)$$

Where: x_i is the disease severity expressed in percentage at i^{th} observation, t_i is time (days after planting) at the i^{th} observation and n is total number of days disease was assessed.

2.5. Phenological Parameters

Plant height (cm): Plant height was measured in centimeters two weeks after pollen shed had ceased, as the distance from the soil surface to the base of lowest tassel branch of 10 plants in the middle two rows.

Days to physiological maturity: Days to maturity of 10 plants from the two middle rows of each plot were recorded as the number of days from emergence to when 50% of the plants in a plot formed a black layer at the tip of each kernel on the ears.

2.6. Grain Yield (GY) and Thousand Seed Weight

Grain yield (kg ha^{-1}): At maturity, the yields of the 10 maize varieties were harvested manually from the two middle rows of each plot. The yield from the two rows was converted into kg per hectare (kg ha^{-1}).

Thousand seed weight (TSW) (g): One thousand randomly taken seeds from each plot were weighed separately and thousand seed weight was reported in grams.

2.7. Data Analysis

Analysis of variance (ANOVA) was conducted for GLS severity, AUDPC and infection rate at all plant growth stages, and yield data were subjected to analysis of variance, and means were compared using least significant difference (LSD) at $p \leq 0.05$ level of significance and SAS Version 9.2 (SAS, 2008) software was employed for the analysis. To determine the disease progress rate, a logistic growth model, ln[x/ (1-x)], (Vander Plank, 1963) was used to estimate the disease progression. The transformed data were regressed over time (DAP) to determine the disease progress rate. The AUDPC values and disease progress rate (r) were calculated for each tested variety and data were analyzed by analyses of variance. The disease progress rates (r) were calculated based on the linearized logistic model (Vander Plank, 1963) and the calculated values were analyzed by using SAS Version 9.2 as follows:

$$r = \frac{\left(\mathrm{Ln}\frac{X}{1-X}\right) - \left(\mathrm{Ln}\frac{X_o}{1-X_o}\right)}{t} \quad (4)$$

Where: r = disease progress rate, X_o = initial disease severity, X = final disease severity, t = the duration of the epidemic and Ln = Natural logarithm. The two locations were considered as different environments because of heterogeneity of variance as tested Bartlett's test (Gomez and Gomez, 1984) and F-test was significant for grey leaf spot reaction on maize varieties under field conditions studied. Thus, the data were not combined for analyses.

Correlation analysis was performed using SAS Version 9.2 to determine relationship among disease assessment parameters, such as disease severity, area under disease progress curve (AUDPC) and infection rate (r) and yield and thousand seed weight.

3. Results

3.1. Disease Development on Maize Varieties under Field Conditions

3.1.1. Grey leaf spot (GLS) severity

Typical grey leaf spot (GLS) symptoms appeared on the highly susceptible variety earlier than on the improved maize varieties at both locations in 2014 and 2015 cropping seasons. Different levels of grey leaf spot severities (as percent leaf area diseased) were recorded on the different maize varieties tested under natural infections. The mean disease severity in the two cropping seasons significantly ($p \leq 0.05$) differeed among the tested varieties. The varieties more or less showed differential responses to the disease.

The mean initial and final disease severity in the two cropping seasons were significantly ($p \leq 0.05$) different among the varieties in both locations (Tables 2 and 3). The lowest mean initial and final disease severity was recorded for both improved and susceptible maize varieties at both locations in 2014 and 2015 cropping seasons. Disease severity during initial assessment varied significantly ($P \leq 0.05$) among the varieties in two seasons of testing at Jimma. Higher initial severities of 24.5 PSI on the variety BH-543 and 28.12 PSI on the susceptible check variety, Local-K, were recorded in 2014 and 2015 cropping seasons at Jimma, respectively, while it was much lower on varieties BH-660 (11.33 PSI), BH-670 (12.67 PSI), Kuleni (12.67 PSI) and BH-540 (13.67 PSI) in 2014 and BH-660 (12 PSI), BH-670 (13.67 PSI), Kuleni (15.5 PSI) and Morka (16.12 PSI) in 2015 cropping season at Jimma. However, there was no significant difference between the four varieties in initial disease severity in 2014; and also there was no significant difference among the three varieties in initial disease severity in 2015 at Jimma.

The susceptible check variety, Local-K, had no significant difference from six of the varieties other than BH-670, BH-660, Kuleni, BH-140, BH 540, and Morka in initial disease severity in 2014, and Local-K had also no significant difference from any one of the varieties other than BH-543 in initial disease severity in 2015 at Jimma (Tables 2 and 3). The level of initial disease severity on the variety BH-543 was 24.5 PSI, which was even higher than the susceptible check Local-K in 2014 and lower than the susceptible check variety, Local-K, at Jimma, in 2015.

Disease severity at final assessment near crop physiological maturity was also significantly ($P \leq 0.05$) different among the varieties in 2014 and 2015 at Jimma. Lower final disease severities of 37.33 and 39.5

PSI were recorded on the variety BH-660 in two cropping seasons (2014 and 2015) and the variety BH-670, which had 49.12 PSI and 44 PSI final disease severity at Jimma in two seasons of testing, respectively (Table 3). The levels of final disease severities on the varieties BH-660 and BH-670 were significantly different from that of the other remaining tested varieties; these varieties showed similar reaction at their early growth stages in having significantly lower initial GLS severities. The initial disease severity on the variety BH-670 was slightly higher (13.67PSI) than BH-660 (12 PSI), and was significantly different from the susceptible check, Local-K, (28.12 PSI) at Jimma in two seasons of testing.

At Hawassa, grey leaf spot severity during initial assessment varied significantly ($p \leq 0.05$) among the varieties (Table 3). Higher initial severities of 25.67 PSI and 25.26 PSI were recorded on the susceptible check variety, Local-K, while it was much lower with values of 10.67 PSI and 10.33 PSI on the variety (BH-660), 11.67 PSI and 12 PSI on the variety BH-670, 12 PSI and 13.5 PSI on the variety (Kuleni), and 14 PSI and 16.33 PSI on the variety BH-140 at Hawassa, in 2014 and 2015 cropping seasons, respectively. However, there was no significant difference between the four varieties (BH-140, BH-540, BH-670 and Kuleni) in initial disease severity in 2014 and, also, there was no significant difference among the three varieties (BH-140, BHQPY*-545 and Morka) in initial disease severity at Hawassa in 2015 cropping season. The susceptible check variety, Local-K, had significant difference from all of the rest varieties except BH-543 in initial disease severity (Table 3).

Disease severity at final assessment near crop physiological maturity was also significantly ($p \leq 0.05$) different among the 12 varieties in the two cropping seasons at Hawassa. Lower final disease severity 38.33 PSI and 35.67 PSI was recorded on varieties BH-660 and BH-670, which had 50 PSI and 43 PSI final disease severity in 2014 and 2015 cropping seasons, respectively (Tables 2 and 3). The levels of final disease severity on BH-660 and BH-670 were significantly different from that of the other varieties tested; these varieties showed more or less similar reaction at their early growth stages in having significantly lower initial severity of the disease. The initial disease severity on the BH-670 variety was also somewhat higher than BH-660, and was significantly different from the susceptible check, Local-K, at Hawassa in 2014 and 2015.

3.1.2. Disease Progress Rates

Disease progress rates and parameter estimates due to grey leaf spot are tabulated hereunder (Tables 2 and 3). The disease progress rates showed variations among the 12 maize varieties used in both locations in 2014 and 2015 cropping seasons. Disease progress rates calculated in the 12 maize varieties ranged from 0.0256 to 0.0489 units' day^{-1} and 0.02613 to 0.04340 units day^{-1} at Jimma in 2014 and 2015, respectively (Table 2 and 3). At Hawassa, the rates were in between 0.0273 to 0.0561 units day^{-1} and 0.0262 to 0.0407 unit day^{-1} in 2014 and 2015 cropping seasons, respectively.

The disease progress rate was relatively higher at Jimma than at Hawassa in 2015 cropping season, while the disease progress rate was also lower at Jimma than at Hawassa in 2014 cropping season. On the varieties BH-540, BH-543, Local-K, BH-661, Gibe-1, Gibe-2 and BH-140 at both locations, the disease progressed faster than in the other five varieties in 2014, whereas in 2015 on the varieties BH-140, Local-K, BH-543, BH-661, Gibe-1 and Gibe-2 in both locations, the disease progressed faster than in the other six varieties. The calculated disease progress rates were significantly different among the maize varieties tested. Even though the maximum AUDPC was observed on Local-K, relatively faster mean disease progress rates, i.e. $r = 0.0561$ and $r = 0.0407$, over the years was observed on the varietiesBH-540 and BH-140 at Hawassa in 2014 and 2015, respectively. On the other hand, the maximum AUDPC was observed on Local-K; however, faster mean disease progress (infection) rate, $r = 0.04340$ over the seasons was observed on the variety BH-540 at Jimma in 2015. The overall mean infection rate on BH-540 and BH-140 was higher than that of the rates on BH-670, BH-660, Kuleni and Morka in both locations (Tables 2 and 3). Disease progress rates of 0.025 and 0.031 units day^{-1} were observed on the varieties BH-660 and BH-670, while the rates were 0.027 and 0.033 units day^{-1} on the varieties BH-660 and BH-670, respectively, although there was significant difference between infection rates on BH-660 and BH-670 as well as Kuleni at Jimma, while no significant difference was observed at Hawassa in 2014 cropping season.

Table 2. Mean initial (PSI_i) and final (PSI_f) severity indices and parameter estimates of grey leaf spot (*C. zeae-maydis*) on 12 maize varieties at Jimma and Hawassa in 2014 main cropping seasons.

Varieties	Jimma in 2014			Hawssa in 2014		
	PSI_{intial}	PSI_{final}	Disease progress rate (Logit days^{-1})	PSI_{intial}	PSI_{final}	Disease progress rate (Logit days^{-1})
Local-K	23.00±1.73^{ab}	84.83±1.44^{a}	0.0489±0.0018^{a}	25.67±0.52^{a}	84.00±0.89^{a}	0.0453±0.0007^{bc}
BH-543	24.50±0.50^{a}	80.00±2.29^{ab}	0.0419±0.0028^{cd}	22.33±0.51^{b}	83.67±2.06^{a}	0.0481±0.0022^{b}
Gibe-2	21.00±2.00^{bc}	79.25±2.16^{bc}	0.0445±0.0024^{bc}	19.00±0.89^{c}	82.12±2.46^{a}	0.0497±0.0036^{b}
BH-140	17.00±2.64^{d}	71.50±6.26^{de}	0.0421±0.0036^{cd}	14.00±2.36^{d}	75.33±1.36^{bc}	0.0490±0.0019^{b}
BH-540	13.67±2.31^{e}	74.00±3.60^{cd}	0.0484±0.0010^{ab}	12.00±1.55^{de}	79.67±1.36^{ab}	0.0561±0.0031^{a}
Gibe-1	22.33±0.57^{a-c}	78.00±0.86^{bc}	0.0418±0.0012^{cd}	20.33±0.51^{bc}	81.00±1.54^{a}	0.0469±0.0020^{b}
BH-661	20.33±0.57^{bc}	75.33±1.57^{b-d}	0.0413±0.0008^{cd}	19.00±0.89^{c}	82.33±1.86^{a}	0.0499±0.0028^{b}
BHQPY*-545	21.00±1.00^{bc}	74.33±2.52^{cd}	0.0399±0.0019^{de}	18.67±1.36^{c}	72.33±2.25^{cd}	0.0406±0.0031^{cd}
Kuleni	12.67±1.53^{e}	55.50±1.50^{f}	0.0359±0.0028^{ef}	12.00±0.89^{de}	49.00±4.47^{e}	0.0325±0.0026^{fg}
Morka	19.60±2.94^{cd}	66.50±3.96^{e}	0.0351±0.0042^{fg}	18.00±2.36^{c}	68.00±4.73^{d}	0.0380±0.0048^{de}
BH-670	12.67±1.53^{e}	49.12±1.75^{g}	0.0317±0.0017^{g}	11.67±1.03^{de}	50.00±2.25^{e}	0.0335±0.0009^{ef}
BH-660	11.33±1.53^{e}	37.33±4.85^{h}	0.0256±0.0033^{h}	10.67±1.03^{e}	38.33±4.50^{f}	0.0273±0.0034^{g}
CV (%)	9.83	4.51	6.25	8.56	4.35	7.35
LSD(0.05)	3.04	5.25	0.0042	2.45	5.19	0.0054

Note: Mean within a column followed by the same letters are not significantly different from each other according to LSD at 5% probability level. LSD = Least Significant Difference, CV = Coefficient of Variation. Initial and final disease severity (PSI) of grey leaf spot.

Table 3. Mean initial (PSI_i) and final (PSI_f) severity indices and parameter estimates of grey leaf spot (*C. zeae-maydis*) on 12 maize varieties at Jimma and Hawassa in 2015 main cropping seasons.

Varieties	Jimma in 2015			Hawassa in 2015		
	PSI_{intial}	PSI_{final}	Disease progress rate (Logit days^{-1})	PSI_{intial}	PSI_{final}	Disease progress rate (Logit days^{-1})
Local-K	28.12±3.40^{a}	81.83±1.44^{a}	0.0407±0.0036^{ab}	25.26±1.10^{a}	78.12±0.78^{a}	0.0393±0.0012^{ab}
BH-543	26.67±2.00^{ab}	78.33±4.31^{ab}	0.0385±0.0015^{b}	23.67±2.00^{ab}	74.83±0.76^{ab}	0.0377±0.0025^{b}
Gibe-2	24.12±0.76^{b}	76.25±2.16^{bc}	0.0385±0.0034^{ab}	21.16±0.76^{cd}	73.08±3.76^{b}	0.0386±0.0026^{ab}
BH-140	18.67±1.53^{cd}	70.00±1.73^{d}	0.0387±0.0030^{ab}	16.33±1.15^{g}	69.12±0.28^{c}	0.0407±0.0013^{a}
BH-540	19.50±0.87^{c}	73.67±0.57^{cd}	0.0434±0.0019^{a}	19.00±2.64^{de}	67.83±2.75^{c}	0.0367±0.0039^{bc}
Gibe-1	24.50±2.78^{b}	75.00±0.86^{bc}	0.0371±0.0025^{b}	21.50±2.78^{bc}	71.50±3.50^{bc}	0.0370±0.0023^{b}
BH-661	19.83±0.76^{c}	73.67±1.52^{cd}	0.0404±0.0018^{ab}	18.83±1.26^{ef}	55.12±1.61^{e}	0.0278±0.0023^{e}
BHQPY*-545	18.42±0.80^{c-e}	73.33±2.08^{cd}	0.0417±0.0024^{ab}	16.75±1.14^{fg}	60.50±1.32^{d}	0.0338±0.0018^{cd}
Kuleni	15.50±0.50^{ef}	52.50±1.50^{f}	0.0299±0.0004^{cd}	13.50±0.50^{hi}	46.83±1.04^{f}	0.0288±0.0002^{e}
Morka	16.12±2.36^{def}	56.83±4.36^{e}	0.0321±0.0051^{c}	14.92±0.72^{gh}	56.12±3.88^{e}	0.0332±0.0034^{d}
BH-670	13.67±0.28^{fg}	44.00±4.27^{g}	0.0267±0.0028^{d}	12.00±0.00^{ij}	43.00±1.00^{g}	0.0285±0.0006^{e}
BH-660	12.00±1.00^{g}	39.50±1.00^{h}	0.0261±0.0020^{d}	10.33±0.57^{j}	35.67±2.08^{h}	0.0262±0.0005^{e}
CV (%)	9.37	3.83	8.02	7.44	3.57	5.11
LSD(0.05)	3.14	4.29	0.0049	2.24	3.69	0.0029

Note: Means within a column followed by the same letters are not significantly different from each other according to LSD at 5% probability level. LSD = Least Significant Difference, CV = Coefficient of Variation. Initial and final disease severity (PSI) of grey leaf spot.

3.1.3. Area under Disease Progress Curve (AUDPC)

AUDPC was computed from severity data and showed highly significant ($p \leq 0.01$) difference among the tested 12 maize varieties at both locations in 2014 cropping season (Figures 1 and 2). In 2014 cropping season, AUDPC varied between 1426.67 and 3281.67%-days in Jimma, whereas the AUDPC values varied between 1476.67 and 3225%-days in Hawassa. The highest (3281.67%-days) AUDPC value was calculated for the Local-K, which is considered as a more susceptible variety in Jimma, while 3225%-day was calculated for the same Local-K in Hawassa. Since the lowest AUDPC value was calculated for the variety BH-660, followed by BH-670 and Kuleni varieties, these varieties are considered as resistant to GLS at both locations in the two testing seasons (Figures 1 and 2).

There was also highly significant ($p \leq 0.01$) difference in AUDPC values computed from severity data among the tested 12 maize varieties at both locations in 2015 cropping seasons (Figures 3 and 4). In 2015 year, AUDPC values varied between 1176.17and 3031.67%-days at Jimma, whereas the AUDPC values varied between 1226.67 and 2875%-days in Hawassa. The highest (3031.67%-days) AUDPC value was calculated for the Local-K that was considered as a more susceptible variety at Jimma than at Hawassa, while 2975%-days were calculated for the Local-K at Hawassa. Since the lowest AUDPC value was calculated for BH-660, followed by the varieties BH-670 and Kuleni, they were considered as resistant to the disease at the two locations in the two testing seasons. All varieties that were considered as susceptible resulted in consistently higher area under disease progress curve at Jimma than at Hawassa during the two testing seasons. The result of this analysis in line with those data obtained from different similar assessment. Overall, results of the two season experiments indicated a difference but stable reaction by the varieties to natural infection by grey leaf spot at Hawassa (south Ethiopia) and Jimma (southwest Ethiopia).

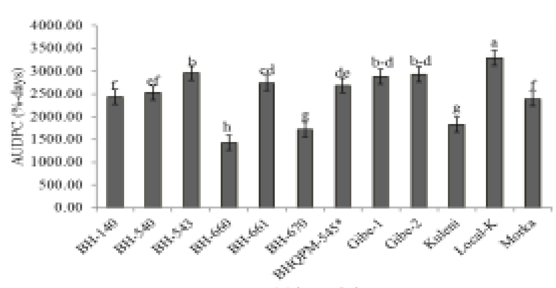

Figure 1. AUDPC values for maize grey leaf spot on 12 maize varieties tested in 2014 cropping season in Jimma, southwestern Ethiopia.

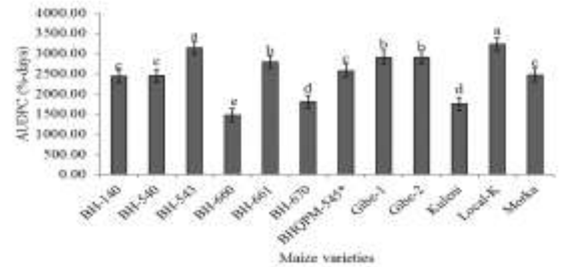

Figure 2. AUDPC values of grey leaf spot on 12 maize varieties tested in 2014 cropping season in Hawassa, south Ethiopia.

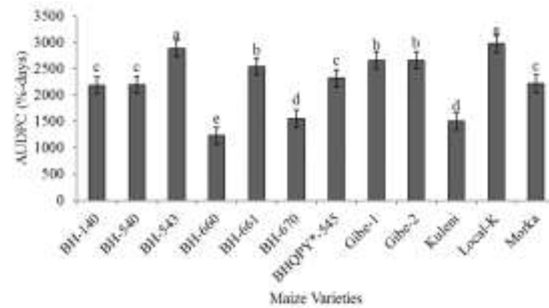

Figure 3. AUDPC values of maize grey leaf spot on 12 maize varieties tested in 2015 cropping season in Jimma, southwestern Ethiopia.

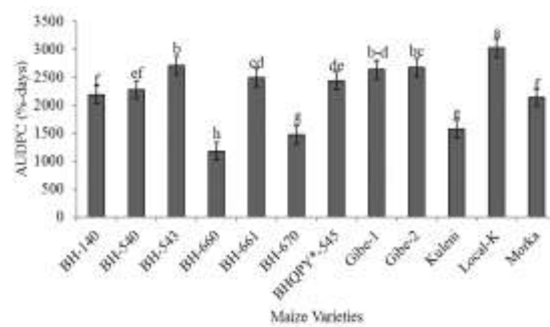

Figure 4. AUDPC values of maize grey leaf spot on twelve maize varieties tested in 2015 cropping season in Hawassa, southern Ethiopia.

3.1.4. Disease Progress Curve

Disease onset (DO) of grey leaf spot appeared at Jimma and Hawassa early at 50 and 52 days after planting, respectively, in 2014 cropping seasons. However, disease appearance at Jimma and Hawassa also appeared at 54 and 60 days after planting, respectively, in 2015 cropping season. The dry weather at Hawassa at planting time most probably delayed the onset of grey leaf spot there (Data was not shown). The disease progress curve of grey leaf spot was sketched coherently for 12 maize varieties tested at both locations (Figures 5 – 8). Each curve for 12 maize varieties revealed that disease severity progressed increasingly starting from the onset to the final severity records at both locations during the study periods.

The four disease progress curves for 12 maize varieties tested also indicated that the disease progress was not similar for all improved and susceptible check maize varieties used. Disease severity in Local-K, BH-543, Gibe-1 and Gibe-2 followed relatively high progressive curve trends and displayed the highest levels of grey leaf spot severity in the two cropping seasons at Jimma. Disease severity in BH-540, BH-140, BH-661 and BHQPY*-545 followed roughly similar curves and lied at intermediate levels of grey leaf spot severity, whereas disease progress curves of BH-660, BH-670, Kuleni and Morka displayed the lowest levels of grey leaf spot severity at Jimma at different days after planting in the two testing seasons (Figures 5 and 7).

On the other hand, disease development also differed markedly among maize varieties at Hawassa. Disease severity in Local-K, BH-543, Gibe-1, and Gibe-2 followed relatively high progressive curve and displayed the highest levels of grey leaf spot severity. Disease severity in BH-540, BH-140, BH-661, BHQPY*-545 and Morka followed medium curves and lied intermediate levels of grey leaf spot severity while the varieties BH-660, BH-670 and Kuleni had low levels of grey leaf spot infection at Hawassa at different days after planting in 2014 and 2015 cropping seasons (Figures 6 and 8).

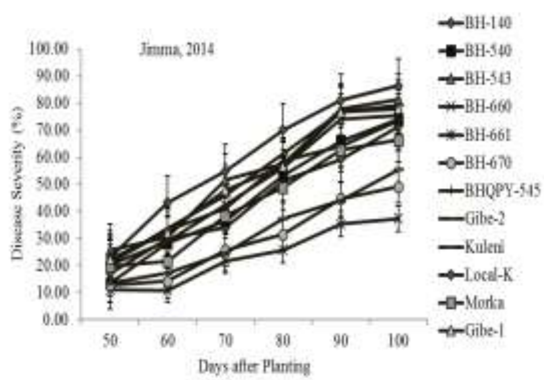

Figure 5. Disease progress curve on 12 maize varieties at Jimma in 2014 main cropping season.

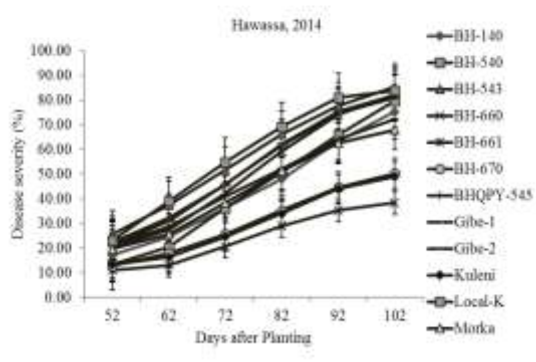

Figure 6. Disease progress curve on 12 maize varieties at Hawassa in 2014 main cropping season.

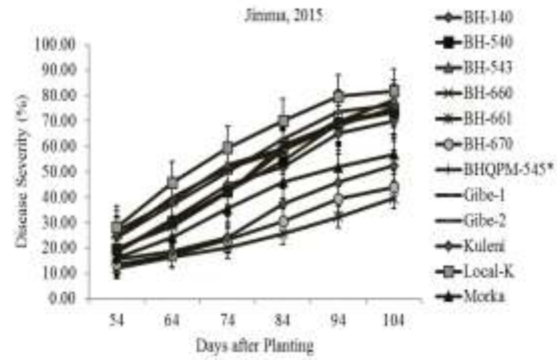

Figure 7. Disease progress curve on 12 maize varieties at Jimma in 2015 main cropping season.

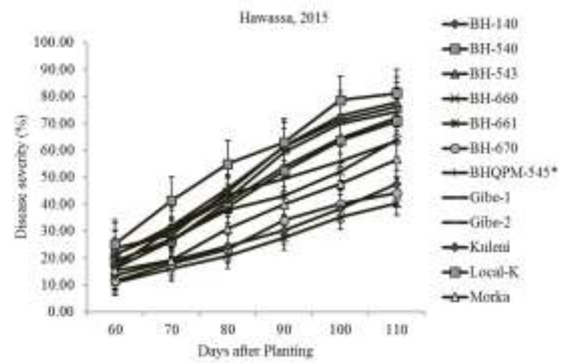

Figure 8. Disease progress curve on 12 maize varieties at Hawassa in 2015 main cropping season.

3.2. Phenological Parameters

The phenological parameters of all tested maize varieties showed a highly significant ($p \leq 0.01$) difference among each other at the two locations in 2014 cropping season (Table 4). The tallest plant heights of 333.3 and 254.33 cm were measured from the variety Morka from the two experimental sites of Jimma and Hawassa, while Gibe-2 with plant heights of 192 and 205.67 cm was the shortest variety at Jimma and Hawassa, respectively, measured in 2014 cropping season. There was also highly significant ($p \leq 0.01$) difference in plant height measured among the tested 12 maize varieties at the two locations in 2015 cropping season (Table 5). The plant heights of all tested maize varieties showed taller appearance at Jimma than at Hawassa in both 2014 and 2015 cropping seasons. The tallest appearance of tested maize varieties at Jimma was due to the prolonged growing season and effect of weather. The prolonged season may lead to delay in maturity and increase in vegetative growth of the maize varieties. At Jimma, the variety Local-K was the latest and the earliest in maturity was BH-660, while at Hawassa the variety Local-K was the latest and the earliest in maturity was BH-660 in the two testing seasons

Table 4. Plant height and days to physiological maturity of 12 maize varieties in Jimma (southwest Ethiopia) and Hawassa (south Ethiopia) in 2014 main cropping season.

Varieties	Jimma in 2014		Hawassa in 2014	
	Plant height (cm)	Days to maturity	Plant Height (cm)	Days to maturity
BH-140	198.90±11.56^{f-h}	147.67±2.25^{fg}	237.67±5.86^{a-d}	148.00±1.78^{d}
BH-540	218.90±17.13^{d-f}	149.67±2.25^{fg}	229.33±5.03^{a-d}	146.00±1.78^{d}
BH-543	229.70±15.82^{c-e}	150.33±2.25^{f}	212.67±8.62^{cd}	148.33±6.77^{d}
BH-660	241.06±3.94^{b-d}	145.00±2.68^{g}	251.67±10.21^{ab}	144.33±2.25^{d}
BH-661	250.40±10.14^{bc}	161.67±3.14^{c}	233.33±7.76^{a-d}	158.00±1.78^{c}
BH-670	257.40±16.05^{b}	161.00±3.22^{d}	256.00±2.64^{a}	157.00±1.78^{c}
BHQPY*-545	195.50±14.23^{gh}	167.67±2.25^{c}	236.33±6.43^{a-d}	165.33±2.25^{b}
Gibe - 1	215.67±11.58^{c-g}	148.00±2.68^{fg}	228.00±5.00^{a-d}	145.33±2.25^{d}
Gibe - 2	191.76±15.33^{h}	162.33±1.86^{d}	205.67±33.65^{d}	160.00±2.68^{bc}
Kuleni	221.80±4.39^{de}	155.00±2.68^{e}	216.00±36.29^{b-d}	150.00±2.68^{d}
Local-K	256.13±2.01^{b}	195.00±2.68^{a}	210.33±37.63^{cd}	183.67±2.25^{a}
Morka	333.27±22.46^{a}	181.00±2.36^{b}	245.33±30.17^{a-c}	179.33±2.73^{a}
CV (%)	5.17	1.60	8.27	2.14
LSD(0.05)	20.51	4.32	32.25	5.68

Note: Mean within a column followed by the same letters are not significantly different from each other according to LSD at 5% probability level. CV = Coefficient of variation, LSD = Least significant difference.

Table 5. Plant height and days to physiological maturity of 12 maize varieties in Jimma (southwest Ethiopia) and Hawassa (south Ethiopia) in 2015 cropping season.

Varieties	Jimma in 2015		Hawassa in 2015	
	Plant height (cm)	Day to maturity	Plant Height (cm)	Day to maturity
BH-140	204.90±12.92^{e-f}	145.67±2.52^{g}	232.67±5.86^{a-c}	151.00±2.00^{d}
BH-540	224.90±19.15^{de}	147.67±2.52^{f}	224.33±5.03^{a-c}	149.00±2.00^{d}
BH-543	235.70±17.68^{cd}	148.33±2.52^{f}	207.67±8.62^{bc}	151.33±7.57^{d}
BH-660	201.50±15.91^{fg}	143.00±3.00^{g}	205.33±37.63^{c}	147.33±2.51^{d}
BH-661	247.07±4.41^{bc}	159.67±3.51^{d}	228.33±7.76^{a-c}	161.00±2.00^{c}
BH-670	256.40±11.34^{b}	159.00±3.60^{d}	231.33±6.43^{a-c}	160.00±2.00^{c}
BHQPY*-545	263.40±17.94^{b}	165.67±2.52^{c}	246.67±10.21^{a}	168.33±2.51^{bc}
Gibe - 1	221.67±12.95^{d-f}	146.00±3.00^{fg}	223.00±5.00^{a-c}	148.33±2.51^{d}
Gibe - 2	197.76±17.14^{g}	160.33±2.08^{c}	200.67±33.65^{c}	163.00±3.00^{bc}
Kuleni	227.80±4.91^{cd}	153.00±3.00^{e}	211.00±36.29^{b-c}	153.00±3.00^{d}
Local-K	262.40±2.25^{b}	193.00±3.00^{a}	251.00±2.64^{a}	186.67±2.51^{a}
Morka	339.27±25.12^{a}	179.00±2.64^{b}	240.33±30.17^{ab}	182.33±3.05^{a}
CV (%)	5.04	1.61	8.46	2.09
LSD(0.05)	20.51	4.32	32.25	5.68

Note: Mean within a column followed by the same letters are not significantly different from each other according to LSD at 5% probability level. CV = Coefficient of variation, LSD = Least significant difference.

3.3. Grain Yield (GY) and Thousand Seed Weight

Grain yield showed a highly significant ($P \leq 0.01$) difference among the 12 tested maize varieties in both locations in 2014 cropping season (Table 6). At Hawassa, the mean grain yield and thousand seed weight were significantly ($p \leq 0.05$) greater than that of Jimma when mean disease severity was significantly ($p \leq 0.05$) lower in the 2014 main cropping season. This was because the disease at Jimma started early and the growth stage of the crop was followed by very fast disease progress before the crop reached dent or physiological maturity stage, whereas at Hawassa highest grain yield and thousand seed weight were obtained in the two main cropping seasons since the disease symptoms were observed later and the development of the disease was slow and reached maximum when the crop reached maturity stage. Therefore, the disease effect on grain yield was relatively higher in Jimma than in Hawassa during 2014 cropping season.

The lowest grain yield and thousand seed weight (TSW) (3514.7 kg ha^{-1}, 367.77 g), (3570.2 kg ha^{-1}, 371.46 g) and (4018 kg ha^{-1}, 312.87 g) was measured on BH-543, Gibe-2 and Local-K maize varieties, respectively, at Jimma in 2014 cropping season, whereas at Hawassa lowest grain yield and thousand seed weight (5101.9 kg ha^{-1}, 1552.33 g), (5393.2 kg ha^{-1}, 1530.62 g) and (5412.9 kg ha^{-1}, 1401.67 g) was measured on Local-K, Gibe-2 and BH-543, respectively, in 2014 main cropping season. Grain yields of the relative susceptible varieties BH-543, Gibe-2 and Local-K were generally lower in Jimma than in Hawassa in both 2014 and 2015 main cropping seasons. Both BH-543 and Gibe-2 were previously released as moderately resistant varieties to the disease. But, in the present study they were considered as susceptible varieties with the lowest grain yield in the two locations.

The highest grain yields of 5791.8 kg ha^{-1}, 5750.9 kg ha^{-1}, 5620.6 kg ha^{-1} and 5612.7 kg ha^{-1} along with thousand seed weights of (455.83 g, 412.13 g, 406.93 g and 429.57 g were obtained from BH-660, BH-670, Kuleni and Morka maize varieties, respectively, at Jimma in 2014 cropping season, while at Hawassa, the highest grain yields and the corresponding thousand seed weights (7284.8 kg ha^{-1}, 1672.67 g), (7100 kg ha^{-1}, 1605.67 g), (6234.6 kg ha^{-1}, 1572.33 g) and (5787.1 kg ha^{-1}, 1578.33 g) were recorded on BH-660, BH-670, Morka and Kuleni maize varieties, respectively, in 2014 main cropping season.

There was also highly significant ($p \leq 0.01$) difference in grain yield among the 12 tested maize varieties in both locations (Hawassa and Jimma) in 2015 cropping season (Table 7). At Hawassa, the mean grain yield and thousand seed weight was significantly ($p \leq 0.05$) greater than that of Jimma when mean disease severity was significantly ($p \leq 0.05$) lower in 2015 cropping season, whereas the highest grain yield and thousand seed weight were obtained at Hawassa when the disease symptoms were observed later and the development of the disease was slow and reached maximum when the crop reached at maturity stage. Therefore, the disease effect on grain yield was also relatively higher at Jimma than at Hawassa in 2015 testing season.

The lowest grain yields along with thousand seed weights (3760.2 kg ha^{-1}, 317.87 g), (3524.7 kg ha^{-1}, 372.77 g) and (4028 kg ha^{-1}, 376.47 g) were measured on BH-543, Gibe-2 and Local-K maize varieties, respectively, at Jimma in 2015 cropping season, whereas lowest grain yields along with thousand seed weights (5383.2 kg ha^{-1}, 1396.67 g), (5402.9 kg ha^{-1}, 1500.33 g) and (5545.9 kg ha^{-1}, 1488.33 g) were measured on BH-543, Local-K, and Gibe-2, respectively, at Hawassa in 2015 cropping season. Yields of the relative susceptible varieties BH-543, Gibe-2 and Local-K were also generally low at Jimma than at Hawassa in 2014 and 2015 cropping seasons. The highest grain yields along with thousand seed weights (5801.8 kg ha^{-1}, 460.83 g), (5760.9 kg ha^{-1}, 434.57 g), (5630.6 kg ha^{-1}, 411.93 g) and (5099.9 kg ha^{-1}, 410.43 g) were measured on BH-660, BH-670, Kuleni and Morka maize varieties, respectively, at Jimma in 2015 cropping season, while highest grain yields along with thousand seed weights (7274.8 kg ha^{-1}, 1667.67 g), (7090 kg ha^{-1}, 1600.67 g), (6224.6 kg ha^{-1}, 1573.33 g) and (6091.9 kg ha^{-1}, 1568.33 g) were measured on the maize varieties BH-660, BH-670, Morka and Kuleni, respectively, at Hawassa in 2015 cropping season.

Overall, the grain yield obtained from Hawassa experimental site was even higher than that of Jimma in the two cropping seasons. Therefore, variety reaction to disease severity was affected by locations. This means that a variety that was considered as higher or lower yielder in one location acted differently in other locations, indicating there were differences in the reactions of the varieties across locations.

3.4. Correlation Analysis among Disease Variables, Yield and Phenological Parameters

Relationship among the various grey leaf spot evaluations with grain yield and growth parameters at Jimma and Hawassa in 2014 and 2015 cropping seasons were determined (Table 8). The interactions between parameters were generally the same in both locations. In both sites, all yield component and growth parameters, i.e. thousand seed weight, plant height and days to physiological maturity were positively correlated with grain yield at Jimma and negatively correlated with grain yield at Hawassa. On the contrary, all the disease parameters were non-significant and were positively correlated with grain yield at Jimma and non-significant negatively correlated with grain yield at Hawassa. Furthermore, disease parameters, i.e. initial and final percent severity indices, area under disease progress curves (AUDPC) and disease progress rates (r) were highly significant and positively associated with each other in both locations. Thousand seed weight, plant height and days to maturity were also non-significant and positively correlated to each other except that plant height was significant and positively associated with both thousand seed weight and days to physiological maturity at Jimma, while at Hawassa it was non-significant and negatively correlated with each other except thousand seed weight that was non-significant and positively associated with both plant heights and days to physiological maturity. Generally thousand seed weight was weakly correlated with all parameters at Hawassa experimental site and significantly and positively correlated with disease progress rate and plant height at Jimma.

Table 6. Grain yields and thousand seed weights of 12 maize varieties tested at Jimma (southwest Ethiopia) and Hawassa (south Ethiopia) Ethiopia in 2014 cropping season.

Varieties	Jimma in 2014		Hawassa in 2014	
	Yield (kg ha^{-1})	TSW(g)	Yield (kg ha^{-1})	TSW (g)
BH-140	4189.40±716.08^{b-d}	395.07±51.92^{bc}	5710.20±896.83^{b}	1505.33±48.01^{ab}
BH-540	4892.30±278.98^{a-c}	383.67±36.05^{bc}	5778.40±614.86^{b}	1557.33±71.23^{ab}
BH-543	3514.70±336.48^{d}	367.77±38.61^{cd}	5412.90±348.62^{b}	1401.67±32.33^{b}
BH-660	5791.80±446.08^{a}	455.83±26.84^{b}	7284.80±158.49^{a}	1672.67±92.50^{a}
BH-661	4909.60±365.67^{ab}	399.30±35.25^{a-c}	5584.10±1328.72^{b}	1530.33±13.01^{ab}
BH-670	5750.90±751.53^{a}	412.13±57.03^{a-c}	7100.00±1098.50^{a}	1605.67±233.35^{a}
BHQPY*-545	5093.20±401.24^{ab}	405.43±16.52^{abc}	5723.41±500.72^{b}	1573.33±132.19^{ab}
Gibe - 1	4182.70±260.53^{b-d}	392.87±40.72^{bc}	5555.90±452.57^{b}	1493.33±53.91^{ab}
Gibe - 2	3750.20±712.79^{d}	371.46±26.05^{bc}	5393.20±327.11^{b}	1530.67±13.01^{ab}
Kuleni	5620.60±763.49^{a}	406.93±10.42^{a-c}	5787.10±917.60^{b}	1578.33±127.75^{ab}
Local-K	4018.00±422.81^{cd}	312.87±5.95^{d}	6101.90±1315.45^{ab}	1552.33±120.38^{ab}
Morka	5612.70±656.69^{a}	429.57±49.59^{bc}	6234.60±660.77^{ab}	1572.33±73.00^{ab}
CV (%)	12.09	8.73	12.15	6.88
LSD(0.05)	978.27	58.31	1228.9	180.37

Note: Means within a column followed by the same letters are not significantly different from each other according to LSD at 5% probability level. TSW = Thousand seed weight, CV = Coefficient of variation, LSD = Least significant difference.

Table 7. Grain yields and thousand seed weights of 12 maize varieties tested at Jimma (southwest Ethiopia) and Hawassa (south Ethiopia) in 2015 cropping season.

Varieties	Jimma in 2015		Hawassa in 2015	
	Yield (kg ha^{-1})	TSW(g)	Yield (kg ha^{-1})	TSW(g)
BH-140	4199.40±800.60^{b-d}	404.30±39.41^{a-c}	5700.20±896.83^{b}	1525.33±34.78^{ab}
BH-540	4902.90±311.91^{a-c}	400.07±58.04^{bc}	5768.40±614.86^{b}	1567.33±73.00^{ab}
BH-543	3760.20±796.93^{d}	317.87±6.65^{d}	5383.20±327.11^{b}	1396.67±32.33^{b}
BH-660	5801.82±498.74^{a}	460.83±30.01^{a}	7274.80±158.49^{a}	1667.67±92.50^{a}
BH-661	4919.60±408.84^{a-c}	397.87±45.52^{bc}	5574.10±1328.72^{b}	1557.33±71.23^{ab}
BH-670	5760.90±840.23^{a}	434.57±55.45^{ab}	7090.00±1098.49^{a}	1600.67±233.35^{a}
BHQPY*-545	5622.7±734.21^{a}	417.13±63.75^{a-c}	5777.10±917.60^{b}	1525.67±34.78^{ab}
Gibe - 1	4192.70±291.28^{b-d}	388.67±40.30^{bc}	5713.40±500.72^{b}	1547.00±120.38^{ab}
Gibe - 2	3524.70±376.20^{d}	372.77±43.17^{cd}	5545.90±452.58^{b}	1488.33±53.91^{ab}
Kuleni	5630.6±853.62^{a}	411.93±11.65^{a-c}	6224.60±660.77^{ab}	1573.33±127.75^{ab}
Local-K	4028.00±472.72^{cd}	376.47±29.16^{bc}	5402.90±348.62^{b}	1500.33±48.01^{ab}
Morka	5099.90±453.12^{ab}	410.43±18.47^{a-c}	6091.90±1315.45^{ab}	1568.33±132.19^{ab}
CV (%)	12.05	8.62	12.17	6.90
LSD(0.05)	978.52	58.31	1228	180.40

Note: Mean within a column followed by the same letters are not significantly different from each other according to LSD at 5% probability level. TSW = Thousand seed weight, CV = Coefficient of variation, LSD = Least significant difference.

4. Discussion

Maize is commonly produced by small-scale farmers throughout the world and its production is highly affected by grey leaf spot (GLS). Because chemical control of grey leaf spot is not practical and economic in maize production areas, adoption of resistant hybrid(s) has been established as a cost-effective means of managing grey leaf spot (Ininda *et al.*, 2007).

The present research confirmed different maize varieties reacted differently to grey leaf spot in different locations in the different cropping seasons. This work is related with the findings of Wang *et al.* (1998) and Dunkel and Levy (2000) who reported that there is evidence that the virulence of *C. zeae-maydis* is changing or that races of the pathogen exist predominantly.

Based on the research results of the present study, the epidemics of grey leaf spot were slightly higher at Jimma than at Hawassa in both 2014 and 2015 cropping seasons. This could be due to variation in altitude and associated amount, distribution and timing of rainfall and the variation in the day temperature. Jimma area had longer extended period of rainfall and more rainy days than Hawassa and mild mean daily temperature (that ranged from 14.1 to 26.3 ^{o}C) and higher relative humidity in the two cropping periods. These weather conditions might have strongly influenced the early initiation and progress of grey leaf spot in the cropping seasons. In line with the current finding, Beckman and Payne (1982) and Rupe *et al.* (1982) reported that grey leaf spot development is favored by extended periods of overcast days, warm temperatures and high relative humidity. High relative humidity, suitable air temperatures, host susceptibility and the presence of a source of inoculum are the conditions necessary to cause widespread and destructive outbreak of grey leaf spot.

Under field conditions, it was also observed that the disease was developed differently on the tested 12 maize varieties in the south and southwest Ethiopia in the two testing seasons. The calculated disease progress rates were significantly ($p \leq 0.05$) different among the 12 maize varieties. Several studies have also shown that environmental factors have tremendous impacts on the rate of within season grey leaf spot development. In Ohio, Denazareno *et al.* (1992) found that the rate of grey leaf spot progress (r) ranged from 0.13 to 0.17 logits per day under favorable conditions for disease development and 0.02 to 0.06 logits per day under less favorable conditions for GLS development. Nutter and Stromberg (1999) also reported a similar result in Iowa where they obtained more or less higher estimates of disease increase with rates of disease development ranging from 0.07 in 1991 (moderately favorable) to 0.28 logits per day in 1992 (extremely favorable). In South Africa, Ward *et al.* (1999) also found apparent infection rates of up to 0.10 logits per day (moderately favorable) up to 0.16 logits per day (highly favorable) during 1991/1992 rain season.

Table 8. Correlation analysis among disease assessments with grain yield, thousand seed weight and phenological parameters at Jimma and Hawassa in 2014 and 2015 cropping seasons.

Parameters	Jimma[a]						
	PSI_f	AUDPC	IR	Yield	TSW	PH	DM
PSI_i	0.81***	0.86***	0.49**	0.12[ns]	0.19[ns]	0.33[ns]	0.37[ns]
PSI_f		0.97***	0.89***	0.16[ns]	0.48[ns]	0.29[ns]	0.33[ns]
AUDPC			0.83***	0.13[ns]	0.45[ns]	0.36[ns]	0.40[ns]
IR				0.11[ns]	0.58**	0.25[ns]	0.25[ns]
Yield					0.28[ns]	0.22[ns]	0.17[ns]
TSW						0.56**	0.32[ns]
PH							0.81***
Parameters	Hawassa[a]						
	PSI_f	AUDPC	IR	Yield	TSW	PH	DM
PSI_i	0.71***	0.87***	0.35[ns]	-0.08[ns]	-0.06[ns]	-0.35[ns]	0.52*
PSI_f		0.94***	0.89***	-0.23[ns]	0.26[ns]	-0.22[ns]	0.23[ns]
AUDPC			0.74***	-0.16[ns]	0.12[ns]	-0.30[ns]	0.35[ns]
IR				-0.22[ns]	0.37[ns]	-0.18[ns]	-0.04[ns]
Yield					-0.10[ns]	-0.46[ns]	0.03[ns]
TSW						0.03[ns]	0.27[ns]
PH							-0.13[ns]

*Note: Initial and final disease severity (PSI) of grey leaf spot, AUDPC = Area under disease progress curve, IR = Infection rate, TSW = Thousand seed weight, PH = Plant height and DM = Days to maturity. **, * and ns correlation is significant at * $p \leq 0.1$, ** $p \leq 0.01$ and *** $p \leq 0.001$ levels and ns = no significant difference, respectively, and [a]/ = Test locations.*

The epidemics of grey leaf spot progress were faster at Jimma than at Hawassa in both 2014 and 2015 main cropping seasons. This might be due to the inconsistency in environmental conditions, production systems and practices that worsened the problem to maximum. Grey leaf spot was also favored by high rainfall and relative humidity, warm temperatures, and the presence of large amounts of inoculum. These current results are in agreement with previous findings that high grey leaf spot severity has been associated with locations and seasons with high precipitation (Ringer and Grybauskas, 1995). Ringer and Grybauskas (1995) also reported the disease components and grey leaf spot progresses under field conditions. These researchers concluded that rainfall and sporulation during early infection cycles had a significant effect on the development of grey leaf spot. They also postulated that early rains created favorable environmental conditions contributing to relatively high numbers of primary lesions that may provide sufficient inoculum to cause subsequent high levels of disease severity. Rupe *et al.* (1982) also observed that high humidity was frequent in the two-week period prior to large increase in GLS severity.

In the present study, relatively the highest grain yield losses and the smallest thousand seed weight were observed at Jimma in two testing seasons. This was because the disease started early at Jimma and the growth stage of the crop was followed by very fast disease progress before the crop reached dent stage. This present investigation is in line with the work of Ward *et al.* (1999) and Dagne *et al.* (2001) who stated that the extent of damage is dependent on hybrid and on environmental conditions. Increased incidence of grey leaf spot in Africa has been associated with continuous cultivation of maize, and use of susceptible maize cultivars (Denazareno *et al.*, 1993; Gevers *et al.*, 1994). A study conducted on three commercial varieties, namely BH-660, BH-140 and PHB-3253 in Ethiopia for three consecutive years indicated that grain yield loss ranged from 0 to 36.9%, depending on the time of disease onset, disease severity and maize hybrid's susceptibility and yield potential (Tewabech *et al.*, 2012). This indicates that grey leaf spot could be severe in some favorable seasons causing significant yield losses, even on resistant varieties (Dagne *et al.*, 2004).

High levels of maize residue, moist conditions in the crop canopy, and susceptible hybrids are all factors that can contribute to yield loss caused by this grey leaf spot. Fungicide application is costly and not practical in most operations for resource-poor farmers. When maize is planted into no-till fields with infested maize residues remaining on the soil surface and environmental conditions that are favorable for grey leaf spot development, grey leaf spot epidemics usually progress faster and reaches more damaging levels than in the fields where infected residues are either absent or greatly reduced (Denazareno *et al.*, 1992; Ward *et al.*, 1998). To this effect, grey leaf spot epidemics have been frequently reported from different parts of Ethiopia (Jimma, Illubabor, West Wellega, North Omo and the Sidam Zones) in earlier years (Dagne *et al.* 2001; Tewabech *et al.*, 2001; Dagne *et al.*, 2004; Tewabech *et al.*, 2011).

Furthermore, Nzuve *et al.* (2013) stated that grey leaf spot is recognized as one of the yield-limiting diseases with yield losses ranging from 90 to 100% during times of grey leaf spot epidemics. Yield losses associated with grey leaf spot occur when photosynthetic tissue is rendered non-functional due to lesions and/or the blighting of entire leaves. The blighting and premature death of leaves severely limits interception of radiation as well as the production and translocation of photosynthate to developing kernels. This is especially true for the upper eight or nine leaves, which contribute 75 to 90% of the photosynthate to grain filling (Allison and Watson, 1996). Leaves of susceptible hybrids or inbreeds may become severely blighted or killed as early as 30 days prior to physiological maturity (Jenco, 1995; Ward *et al.*, 1996). In Ethiopia, Dagne *et al.* (2004) also found that yield losses due to grey leaf spot on resistant, moderately resistant, and susceptible varieties ranged between 0-14.9, 13.7-18.3 and 20.8-36.9%, respectively, from 2003-2004 cropping seasons in Bako and its nearby areas or vicinities.

The findings of the present study revealed that GLS severity had significant effect on grain yield at Jimma and Hawassa. This variation indicated that the disease development at both locations in the two testing seasons clearly showed that the disease becomes more severe when the susceptible plants are at the tasseling to grain filling stage. In the two testing seasons, disease severity assessment was positive but had non-significant impact on grain yields of all maize varieties at Jimma, while the disease severity was negative but had non-significant impact on grain yield of all maize varieties at Hawassa. This could be due to the higher contribution of the maize leaves in grain filling (converting photosynthetic products to grain). This evidently showed that grain yield loss could be high when disease severity occurs during vegetative and tasseling or silking to grain filling stage and low grain yield loss was found after grain filling stage.

On the other hand, several factors may also contribute to this response, including yield potential of the varieties, growth stage of crops and the ability of leaf blighting to predispose the variety to stalk rots that result in high yield reduction. With reference to the area under disease progress curve at the two locations, AUDPC showed non-significant but negative association with grain yield. The present study indicated that yield reductions due to grey leaf spot were affected by disease severity and AUDPC. Grain yield did not show strong and positive correlations with any of the disease parameters considered.

In general, grain yield was significantly affected by maize variety but no significant difference was observed among varieties for the yield related trait, i.e. thousand seed weight. This means that varieties with low disease severity values had high thousand seed weight. On the other hand, there were strong and positive correlations among all disease parameters, such as GLS severity, AUDPC and infection rate, that were affected by variety. Significant reactions of varieties to the disease indicated that reduction in grain yield due to this disease varied from variety to variety, and that a specific variety showed different amount of yield depression due to disease. Overall, the study showed that grey leaf spot (GLS) attack on maize resulted in significant reduction in grain yield in the absence of any control measure.

Ward *et al.* (1999) suggested management of grey leaf spot through conventional tillage that buries crop residues, crop rotation, fungicides, and utilization of resistant varieties. Fungicides are widely used in maize production (Munkvold *et al.*, 2001) but are too expensive for low income and resource-poor farmers in the tropics (Menkir and Ayodele, 2005). Host plant resistance is found to be the most efficient and cost-efficient means of managing grey leaf spot and preventing leaf blighting (Coates and White, 1994). However, no commercial hybrids with sufficient resistance presently exist in Ethiopia, as they have not been improved for resistance to this specific disease so far (Dagne *et al.*, 2008). Reportedly, breeding programs involved in developing resistance to grey leaf spot are in progress (Latter and Rossi, 1983; Ayers *et al*, 1985). Inbreds and hybrids differs in their level of resistance to the disease, highly resistant lines have not been identified to date. Horizontal resistance appears to be a viable option for improving the levels of grey leaf spot resistance in maize varieties (Ayers *et al*, 1985).

The results of this study demonstrated that the use of resistant maize varieties effectively reduced the disease severity and minimized grain yield losses. This implies that adequate levels of host resistance can prevent reduction in grain yield. The overall results of this study also demonstrated that all the released varieties in two tested locations showed susceptible reaction to grey leaf spot with the exception of hybrid varieties BH-660, BH-670, Kuleni and Morka that were relatively considered as resistant to GLS in 2014 and 2015 cropping seasons.

5. Conclusion

In this field experiment under natural infection, it was identified that the performance of some maize varieties were generally consistent with results of previous seedling test experiments. In this case, moderately resistant varieties, such as Gibe-2 and BH-543, have shown different host susceptible reaction, while the susceptible variety, such as Local-K under field conditions, have shown similar host susceptible reaction. On the optimistic side, the varieties BH-660, BH-670, Kuleni (improved open pollinated variety) and Morka (improved open pollinated variety) showed promising resistant reaction to grey leaf spot in the field.

The current results also designated that the maize varieties, such as BH-660 and BH-670, were considered as resistant under field conditions. Under field condition, grey leaf spot was strongly affected by different resistant levels of maize varieties and difference in the environmental conditions. Disease pressure had strong negative impact on grain-filling, which resulted in large amount of yield losses on susceptible maize varieties across locations. Therefore, understanding relevant variables could play an important role in the management of this major disease of maize in Ethiopia. Ethiopia is a center of diversity for maize; various sources of resistant varieties could be obtained against grey leaf spot through resistant breeding programs.

In conclusion, the present research confirmed that, under field conditions, different maize varieties responded differently to grey leaf spot and the disease severity was strongly affected by the use of different resistance levels of maize varieties and difference in the environmental conditions. It is therefore, promising to use the two maize varieties, such as BH-660 and BH-670, were considered as resistant under field conditions and that are recommended to be used by farmers in south and southwest Ethiopia as they had showed good performance such as moderate resistance to grey leaf spot, vigorous growth and high grain yield under field condition in two seasons of testing. Further studies on the reaction of maize varieties to grey leaf spot should continue to identify and exploit genotypic differences among lines; resistant lines could be exploited by breeders to identify and develop cultivars with high degree of resistance so that they could be used either directly for production or indirectly as parents for developing hybrids.

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