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Controlling of Maize Grey Leaf Spot by Interacting Host Resistance and Fungicide Spray Frequency at Bako, Ethiopia

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Abstract. Grey leaf spot (Cercosporazeae-maydis) is major disease affecting maize in western Ethiopia**.** At Bako National Maize Research Center, a field condition study was conducted to examine the interaction between maize varieties and fungicide spray frequencies on maize GLS and yield, to assess maize yield loss due to GLS, and to conduct cost-benefit analyses of using various fungicide frequencies on maize GLS. The experiment was set up as a factorial combination of 3 maize varieties with 3 tilt 250 EC spray frequencies in a randomized complete block design with 3 replications. The difference between the mean yield of protected plots and unprotected plots of each variety was used to calculate grain yield losses. Finally, correlation and economic analysis were done. Unsprayed variety BH543 had the highest AUDPC value (1676.27%-day), terminal PSI (68.33%), and disease progress rate (0.044500 units-day^{-1}) scored. GLS caused grain yield losses of up to 52.82 % on untreated variety BH543. PSI, AUDPC, incidence and disease progress rate were negatively correlated with yield. The highest marginal benefit (ETB 60486 ha^{-1}), and marginal rate of return (ETB 18.05) were obtained from variety SPRH with once application of propiconazole. Based on current results, one-time tilt 250 EC application was found effective to manage GLS on SPRH1 variety. However, additional experiments should be carried out to verify the current results.

Keywords. Cercosporazeae-maydis, Fungicide spray frequency, Interaction, Varieties, Yield.

1. Introduction

Maize (Zea mays L.) has been chosen as one of the primary national commodity crops to meet the food self-sufficiency program to feed the world's, Africa's, and Ethiopia's rapidly growing populations. It is the most important crop in Ethiopia in terms of production and distribution. Thus, maize is second in area coverage to teff (Eragrostistef) among all cereals, with 2.3 million hectares (17.7% of all cereals) land committed to the crop, but first in productivity (4.7 t ha^{-1}), with total annual production of 10.6 million tons (CSA, 2020). Despite maize's importance as a basic food security crop, Ethiopia's average yield (3.74 t ha^{-1}) remains low when compared to the global average (5.78t ha-1) (FAO, 2020). As a result, the effects of abiotic and biotic factors, as well as insufficient deployment of varieties tolerant or resistant to these scenarios, account for a large amount of the yield gap.Ethiopian maize production is being hampered by biotic stresses like diseases such as Grey Leaf Spot (Cercosporazeae-maydis), Turcicum Leaf Blight (Exserohilumturcicum), Common Leaf Rust (Puccinia sorgi), Maize Streak Virus (Mastre virus), Maize Lethal Necrosis, parasitic weeds (primarily Striga hermontica), and insect pests (such as the maize stem borer, maize weevils (Keno *et al*., 2018). Among fungal disease, GLS, caused by C. zeae-maydis, is a necrotrophic and polycyclic foliar disease of maize that poses a severe challenge in tropical maize production (Renfro and Ullstrup, 1976). This

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pathogen causes the plant to lose a lot of water, resulting in severe leaf blighting and impaired photosynthesis. When C. zeae maydis infects foliar tissue, the plant's capacity to photosynthesize and create byproducts of the process is reduced. This eventually results in undersized ears, reduced grain yield, and maize plant death (Stromberg, 2009). Extreme blighting of the upper eight or nine leaves, which produce 75 to 90% of the photosynthates for grain fill, can cause stalk weakness or potentially infectious stalk rot diseases, which can result in premature stalk death and lodging (Lipps*et al*., 1996: Ward *et al*., 1999).
In Ethiopia, a major epidemic occurred in the early 2000s and made considerable maize grain yield losses 36.9 % and 49.5% and there have been extensively disseminated through severe outbreaks every year, particularly in the warm and humid areas of the country (Tilahun*et al.*, 2012, Negash, 2013). Wegary*et al*. (2004) reported that yield loss due to GLS on resistant, moderately resistant and susceptible varieties was between 0-14.9%, 13.7-18.3%, and 20.8-36.9% respectively during 2003/2004 cropping seasons at Bako areas. Similarly, a study conducted in South Ethiopia in the years 2004-2006 found that GLS caused a 29.5 % yield loss (Tillahun et al., 2012). Under field conditions, BH660 and BH670 were classified as resistant maize varieties, while Gibe2 and BH543 were classified as highly susceptible maize variety (Nega et al., 2018). When conditions were favorable for disease, systemic fungicides were required to prevent high levels of disease and were more cost-effective for growers. Maize production with proper fungicide application boosts producers' profitability and provides consumers with a high-yield, high-quality product. Demethylation inhibitors (DMIs) fungicides, first introduced in the 1970s, have broad-spectrum activity against a wide range of fungal diseases and are approved for use on a wide range of crops (Munkvold et al., 2001). When compared to mancozeb, propiconazole was found to considerably boost maize yields against GLS (Munkvold et al., 2001).
In Ethiopia, even though the effect of propiconazole on grey leaf spot was not done, the research conducted at Ambo area at research station indicated that integration of host resistance with three times sprays of foliar fungicide (propiconazole) protected the maize varieties from high TLB epidemics, increased yield, yield components and maximized marginal benefit (Aliyi*et al.,* 2018). While grey leaf spot is known to be present under field environments little is known about the reaction of several maize varieties and effects of propiconazole to the disease. Moreover, there is limited quantified data that describe the reaction of maize varieties on the disease which calls for investigation. Additionally, application frequency of widely used propiconazole (tilt) fungicide is not yet determined and documented to manage maize grey leaf spot disease in the study area. Therefore, this work is intended to generate comprehensive and informative data on reaction among varieties and the optimum fungicide application frequencies need to enhance maize production and productivity under rain feed condition at Bako. Thus, the objectives of this study were to determine the effect of maize varieties on development of GLS, to evaluate the effect of fungicide frequencies on maize GLS and yield, to examine the integrated effect of maize varieties and fungicide spray frequencies on maize GLSand yield, to assess maize yield loss due to GLS and make cost-benefit analyses of applying different fungicide frequencies on maize GLS.

2. Materials and Methods

2.1. Descriptions of Experimental Site

This experiment was conducted during the 2019/20 main cropping season at Bako National Maize Research Center. The Center is found in East Wollega zone of the Oromia National Regional State, Ethiopia at an altitude of 1650m above sea level (m.a.s.l). It lies between 9°06’ N and 37°09’ E in the sub-humid agro-ecology of the country. The area received rainfall from May to October and maximum rainfall was in the months of July and August. The average annual rainfall was 944.4mm. And also, the minimum and maximum temperatures that the area received were 12.3 and 29.8 °C, respectively. The soil is classified under the Nitosol order. Naturally, this place is exposed to excessive maize foliar disease pressure and in most cases used as a hot spot place to screen new maize genotypes for the most foliar diseases (Wegari*et al*., 2008, Bekeko et al., 2018).

2.2. Experimental Materials

Twelve treatment combinations which comprised of three varieties, three fungicide spray frequencies and one untreated check for each variety were utilized for field experiment. The three mid altitude maize varieties used were BH543, Gibe3 and SPRH1 grow at an altitude of 1000-2000, 1000-1700 and 1500-1800 m.a.s.l, respectively (Table 1). The varieties have potential yields of 85-110, 65-75 and 85-95q ha^{-1}, respectively under good management conditions at research station (Ayana *et al*., 2016). Additionally, those varieties have different reactions to GLS disease under field condition. Thus, BH543 was reported to have highly susceptible reaction (Nega*et al.*, 2018) and Gibe3 and SPRH1 have moderately resistant and resistant reactions to GLS respectively based on previous recommendation (Ayana *et al*., 2016).

2.3. Experimental Design and Management of the Experiment

In a randomized complete block design (RCBD) with three replications, the experiment was set up as a factorial combination of three maize varieties with three propiconazole spray frequencies. Each plot had four rows of three meters each, with inter and intra row spacing of 75 cm and 30 cm, respectively. One plot was $9m^2$ in size (3mx3m). Plots and blocks were separated by 1 m and 1.5 m, respectively. The land used for this experiment was 16.5 m x 47 m in size (775.5 m2). All of the plots were hand-planted. Two seeds were planted per hill, which were then thinned to one at the time of establishment. All other agronomic practices were applied consistently in accordance with research recommendations.

2.4. Inoculum Preparation, Inoculation and Disease Establishment

C. zeae-maydis, the pathogen, was artificially inoculated to all plots using infected leaves obtained from infected maize fields presenting different GLS symptoms in previous years. The infected dried leaves were pulverized into a powder and kept in paper bags at 4 °C until the inoculation. Inocula containing infected leaf powder are effective in producing infections in the field (Wegarietal.,2008).Pinches (Tea spoon amount) of pulverized leaves were sprinkled intothe whorls of the plants' leaves, where they stayed long enough for spore germination to commence.To achieve sufficient infection, inoculation was done under dew circumstances and whenand when ambient temperature was appropriate, with a seven-day intervalcommencing from the 4-6 leaf stage of the plant (Bekekoet al.,2018,Wegari et al.,2008).

2.5. Fungicide Application

Propiconazole (Tilt® 250 EC at 500 ml ha^{-1}) was the fungicide employed in the experiment.500 milliliters dissolved in 200 milliliters of water per hectare. It was sprayed three times, once, twice, and three times at a 10-days interval, starting when lesions were visible on the three to five basal leaves of the susceptible variety or when mature GLS lesions were easily distinguished from those of other foliar diseases of maize, they were gray to tan in color and distinctly rectangular in shape (Tilehun et al., 2012).To reduce differences related to moisture, control plots were treated with water only in the same manner as fungicide-sprayed plots.

Likewise, to minimize the risk of fungicide drift to neighboring plots, the treated plot was bordered by a plastic sheet at the time of fungicide spraying.

2.6. Collected Data

2.6.1. Disease Assessments

Following the commencement of the disease, a disease assessment was carried out in the field. Eight plants from the two central rows were randomly chosen and tagged for subsequent disease assessment.

2.6.2. Latent Period (Disease Appearance Date)

The number of days from disease inoculation to the date on which a clear grey leaf spot lesion was observed on 50% of the plants in a plot was used to calculate the disease appearance date. Because the first inoculation alone may not provide enough infection, the latent period was calculated using the second inoculation.

2.6.3. Disease Incidence

The initial and finale disease incidence were recorded at 49 and 83 DAI, respectively. The number of infected plants in each plot was recorded and their values were converted into percentage of the total number of plants to be inspected (Cooke *et al.,* 2006)

$$Diseaseincidence(\%) = \frac{\text{number of infected plants}}{\text{total number of plants}} * 100$$

2.6.4. Disease Severity

The severity of the disease was evaluated six times at seven-day intervals. It was evaluated using the CIMMYT-recommended 1-5 standard disease rating scale. When obvious genotypic differences for the GLS reaction became visible, scoring began and continued until the leaves began to senescence.

2.6.5. Apparent Infection Rate

The apparent infection rates were used as the coefficient of the regression line, with the six disease severity observations recorded at 7-day intervals. The obtained value and the disease progression rate (r) calculated using the linearized logistic model (Campbell and Madden, 1990, Van der Plank, 1963) were examined using SAS software.

$$\mathrm{r} = \frac{\left(\mathrm{Ln}\,\frac{\mathrm{X}}{1-\mathrm{X}}\right) - \left(\mathrm{Ln}\,\frac{\mathrm{X_o}}{1-\mathrm{X_0}}\right)}{t}$$

Where: r = disease progress rate, Xo = initial disease severity, X = final disease severity
t = the duration of the epidemic and Ln = Natural logarithm.

2.6.6. Area Under Disease Progress Curve (AUDPC)

The AUDPC for each treatment was calculated using the disease percent severity index values.
The area under the disease progress curve was used to calculate the start of the epidemic and the time it took for the disease to reach its peak stage (Campbell and Madden, 1990).

$$\mathrm{AUDPC} = \sum_{n=0}^{n-1} 0.5[(\mathrm{xi}+1+\mathrm{xi})(\mathrm{ti}+1-\mathrm{ti})]$$

Where, xi = is the cumulative percent severity index expressed as a proportion at the i^{th} observation, ti = is the time (days after sowing) at the i^{th} observation, and n = is total number of observations. Since the percent severity index was expressed in percent and time (t) in days, AUDPC values were expressed in %-days

2.6.7. Grain Yield

2.6.7.1. Moisture Contents

A moisture tester tool was used to measure the actual moisture content of the grain from each plot at the harvesting day. The grain sample was poured into the moisture tester's cup until it overflowed. The sample was then poured into the measurement section's center.

2.6.7.2. Yield Per Plot and Per Hectare

Wegari et al. (2015) calculated the total grain yield from the two center rows and adjusted it to 12.5 percent moisture content as follows:

$$\text{Adjusted yield per plot} = \frac{(\mathrm{FW}(100\ -\ \mathrm{AMC})\ *\ 0.8)}{87.5 * \text{plot area}}$$

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Where:
FW = Field weight to be harvested from two central rows of each plot, AMC = Actual moisture content, (Given) = 87.5, 0.8 = Shelling % (Given). Then the yield per plot will be converted into yield per hectare (tonnes ha-^{1}).

2.6.8. Relative Yield Loss
The difference between the mean yield of protected and unprotected plots of the same variety was used to assess grain yield losses. The following formula was used to quantify losses for each of the treatments separately (Tilehun et al., 2012).

$$\mathrm{RYL}(\%) = \frac{(Y1 - Y2)}{Y1} * 100$$

Where: -RYL= relative yield loss,Y1= Mean yield of protected plots (plot with maximum protection, plots that received thrice application of propiconazole fungicide), Y2= Mean yield of unprotected plots (i.e. unsprayed plots)

2.7. Data Analysis
GLS initial and final incidences, terminal percent severity index, AUDPC values, apparent infection rate and yield were analyzed using SAS software version 9.3 in the field (SAS, 2010). At the 5% probability level, the LSD was used to calculate the mean separation. To determine the association between disease parameters with yield and RYL,a correlation analysis was performed using SAS PROC CORR (SAS, 2010).

2.7.1. Partial Budget Analysis
The economic analysis was conducted using the methods given in CIMMYT (1988), which used current market prices for inputs and outputs at planting and harvesting. The mean marketable grain yield of each treatment, the gross benefit (GB) ha-1 (the mean marketable grain yield for each treatment), the field price of three maize varieties seed (BH543, Gibe3, and SPRH1), and different fungicide spray frequencies were employed in the partial budget analysis.

- Adjusted yield (AjY): AjY was the average yield adjusted downward by 10% to reflect the fact that experimental yields are frequently higher than the yields that farmers can expect using the same treatments, thus, farmers' yields are adjusted by 10% less than the research results in economic calculations (CIMMYT, 1988).
- Gross field benefit (GFB): GFB was computed by multiplying field/farm gate price that farmers receive for the maize when they sale it as adjusted marketable maize grain yield.
- Total variable cost (TVC): Prices of grain (Birr t^{-1}) were obtained from local markets and total sale from one hectare was computed. Price of seeds of each variety was collected from local market and farmers union in the localities. Price of propiconazole per liters was assessed and the total price incurred to spray one hectare of maize was also calculated. Other inputs’ cost and production practices such as labor cost, land preparation, planting, weeding, and harvesting were all treated the same way. The cost of spraying those chemicals was calculated. Labor and spray equipment costs were determined using local prices, and cost return and benefit were estimated on a per-hectare basis.
- Net income (NI) or Net benefit (NB): was calculated as the amount of money left when the total variable costs for inputs (TVC) are deducted from the total revenue (TR).

$$NB = TR - TVC$$

- Partial budget analysis or marginal rate of returns were used to conduct cost/benefit analyses for integrated GLS management alternatives (CIMMYT, 1988).
- Marginal rate of return (MRR %): was calculated by dividing change in net benefit by change in total variable cost.

$$\mathrm{MRR}(\%) = \frac{DNI}{DIC}\mathrm{x}100$$

Where, MRR = is marginal rate of returns, DNI = difference in net income compared with control, and DIC = difference in input cost compared with control.

3. Results and Discussion

3.1. Interaction Effects of Maize Varieties and Fungicide Frequencies on Maize Grey Leaf Spot Development

3.1.1. Disease Incidence

There were highly significant ($p<0.01$) difference in disease incidence among varieties and fungicide spray frequencies at both first and final assessment dates.On the BH543, Gibe2, and SPRH1 types, the final incidence differed from the initial incidence by 33.33%, 37.50%, and 37.50%, respectively. Similarly, three times fungicide application decreased the initial incidence by 1.47 rate over unsprayed plot and the final incidence by 1.19 rate over unsprayed plot (Table1). Munkvold et al. (2001) revealed that spraying propiconazole at regular intervals was successful in slowing the development of GLS in maize hybrid varieties.

Table 2. Effects of hybrid varieties and propiconazole spray frequencies on GLS initial and final incidences at Bako during the 2019/20 cropping season.

Variety	Initial incidence (%)	Final incidence (%)
BH543	65.63^{a}	98.96^{a}
Gibe3	58.33^{b}	95.83^{a}
SPRH1	28.13^{c}	65.63^{b}
LSD	5.80	5.34
CV	13.52	7.27
Spray Frequencies		
Unsprayed	61.11^{a}	93.06^{a}
One time	52.78^{b}	90.28^{ab}
Two times	47.22^{c}	86.11^{b}
Three times	41.67^{d}	77.78^{c}
LSD	6.70	6.17
CV	13.52	7.27

Mean values with the same letter within the column are not significantly different at described probability level

3.1.2. Percentage Severity Index (PSI)

At all evaluation dates, there was a highly significant ($p<0.01$) difference in GLS PSI across varieties, fungicide spray frequencies, and their interaction. The current result revealed that, in all varieties, the frequent application of propiconazole reduced the progress of the disease as compared to unsprayed control, but three times application highly reduced the progress of the disease compared to two times and one-time application at all assessment dates. Three times fungicide application reduced the PSI by rate of 1.9, 1.7 and 2 over unsprayed varieties of SPRH1, Gibe3 and BH543 respectively (Table 2). It appears that the genetic resistance potential of the varieties was further boosted by fungicide application as susceptible varieties had high level of disease severity reductions. On the whole, the application of fungicides in different level, three times, two times, and one time have arrested disease development more effectively compared to unsprayed control application. Propiconazole was found to be more effective than mancozeb by Munkvold et al. (2001). The authors also stated that, while the difference was typically not statistically significant, disease severity was numerically reduced with two times propiconazole application compared to single application. On the susceptible, moderately resistant, and resistant varieties, three sprays of propiconazole resulted in a considerable reduction in GLS severity compared to two and one times spray.

Table 3. Interaction effects of maize varieties and propiconazole spray frequencies on PSI of GLS during the 2019/20 main cropping season at Bako, Ethiopia.

Varieties	Spray Frequencies	DAI49	DAI56	DAI63	DAI70	DAI77	DAI83
SPRH1	Unsprayed	30.00^{b}	30.93^{c}	33.67^{c}	35.20^{d}	37.33^{d}	40.00^{e}
SPRH1	One time	21.80^{de}	22.67^{f}	23.93^{e}	25.67^{f}	27.00^{f}	28.33^{g}
SPRH1	Two times	20.00^{f}	20.47^{g}	21.40^{f}	22.53^{g}	24.67^{g}	26.13^{h}
SPRH1	Three times	20.00^{f}	20.00g	20.34^{f}	20.60^{g}	20.80^{h}	21.00^{i}
Gibe3	Unsprayed	30.40^{b}	33.47^{b}	38.00^{b}	42.27^{b}	44.67^{b}	50.00^{b}
Gibe3	One time	30.00^{b}	31.47^{c}	33.33^{c}	35.47^{cd}	37.67^{d}	42.80^{d}
Gibe3	Two times	21.53^{e}	24.67e	28.60^{d}	31.60e	34.00^{d}	35.07^{f}
Gibe3	Three times	20.27^{f}	21.93^{f}	23.67^{f}	26.67^{f}	28.00^{f}	29.33^{g}
BH543	Unsprayed	31.00^{a}	36.40^{a}	44.47^{a}	51.47^{a}	57.47^{a}	68.33^{a}
BH543	One time	30.00^{b}	30.80^{c}	33.47^{c}	37.47^{c}	42.00^{c}	45.20^{c}
BH543	Two times	26.20^{c}	28.93^{d}	32.53^{c}	35.80^{cd}	38.40^{d}	39.20^{e}
BH543	Three times	22.13^{d}	24.80^{e}	27.47^{d}	29.60^{e}	32.20^{e}	34.27^{f}
LSD		0.54	1.41	2.17	2.25	2.30	1.55
CV		1.27	3.05	4.27	4.04	3.84	2.40

Mean values with the same letter within the column are not significantly different at described probability level, LSD=least significant difference, CV=coefficient of variation, PSI= percent severity index, DAI=dates after inoculation

3.1.3. Area Under Disease Progress Curve (AUDPC)

There were highly significant differences ($p<0.01$) between treatment combinations in terms of AUDPC values, according to the two-way interaction of treatment combinations effects of propiconazole application schedules. The maximum AUDPC value (1676.27%-days) was recorded on unsprayed plots of the susceptible variety, BH543, whereas the minimum AUDPC value (717.08%-days) was registered on the resistant variety, SPRH, with three propiconazole applications (Figure 1). Previous works at Bako indicated genotypes considered as susceptible variety had AUDPC values more than resistant genotypes (Tilahun*et al*., 2012). Wegari et al. (2008) also found that susceptible variety had greater area under disease progression curves than resistant varieties. Gray leaf spot pressure was present on susceptible local maize, and high inoculum pressure had a significant impact on disease development and reproduction, in line with Madden's findings (Madden et al., 2007).

Wegari*et al*. (2008) also reported higher values of area under disease progress curves on susceptible varieties than resistant varieties. There was gray leaf spot pressure on the susceptible local maize and high inoculum pressure had major influence on disease development and reproduction in conformity with the findings of Madden (Madden *et al.,* 2007). AUDPC of the varieties Gibe3 that of untreated plot, BH543 treated with one time, Gibe3 treated with one time, SPRH1 that of untreated plot, BH543 treated with two times, Gibe3 treated with two times, BH543 treated with three times spray were recorded with 1390.20, 1269.33, 1220.33, 1204.93, 1178.57, 1030.17, 995.87%-days, respectively. AUDPC of the varieties Gibe3 treated three times, SPRH1 treated one time, and SPRH1 treated twice were scored with 875.47, 870.33, and 784.93 % -days, respectively.

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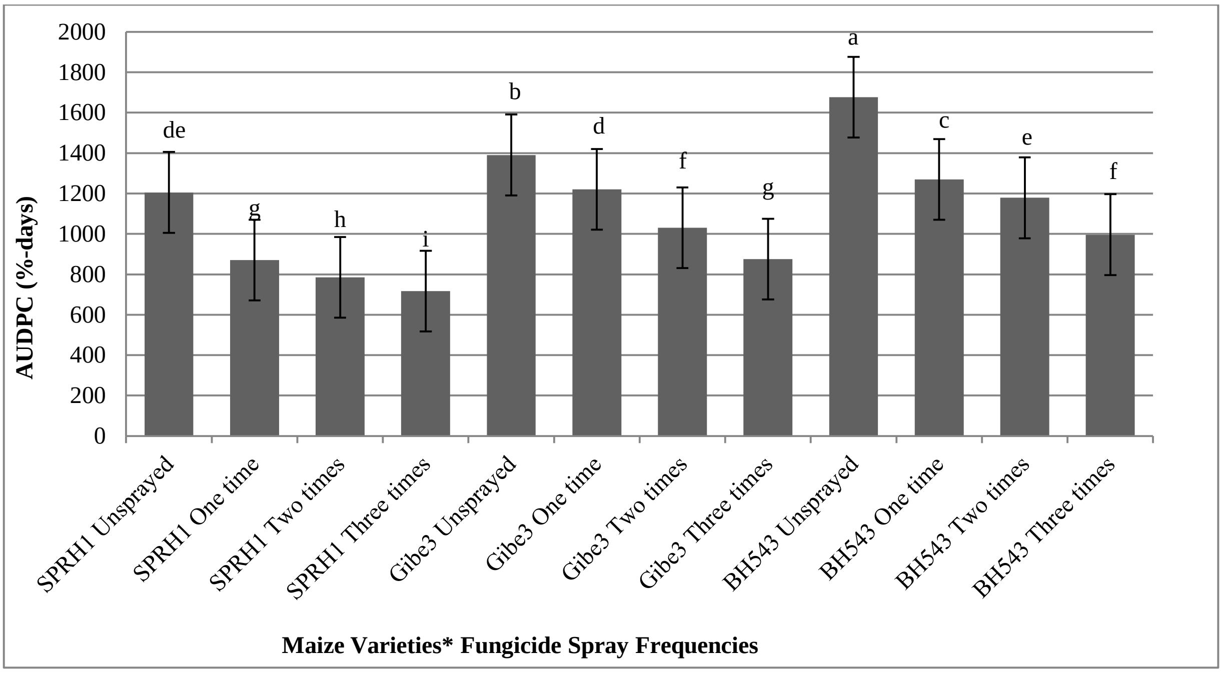

LSD=41.49, LSD=Least significant difference

Figure 1. Effects of hybrid varieties and their interactions with propiconazole spray frequencies on GLS AUDPC values at Bako during the 2019/20 cropping season. Bars with the same letter(s) are not significantly different at p<0.01.

3.1.4. Progress Rate of Grey Leaf Spot on Hybrid Maize Varieties and Fungicide Spray Frequencies

In terms of GLS progress rate, varieties, propiconazole application frequency, and their interactions differed significantly ($p<0.01$).In this study, the apparent infection rate of GLS ranged from 0.001803 to 0.044500 units-day^{-1}. Accordingly, the BH543 variety treated with thrice application of propiconazole fungicide had the fastest disease progression rate (0.044500 units-day-1) and the SPRH1 variety treated with thrice application of propiconazole fungicide had the slowest (0.001803 units-day-1). The disease progress rate of an unsprayed plot of susceptible variety BH543 was 2.524 times faster than a three-time application of propiconazole fungicide, whereas the GLS progress rate of a resistant variety, SPRH1, was 7.222 times faster than a three-time application of the same variety (Figure 2). This indicated that using propiconazole three times slowed the progression of GLS disease. Mengist and Moges (2018) reported apparent infection rate of GLS varying between 0.0315 and 0.0862 units-day^{-1}. In the same way, Nega et al. (2018) reported that apparent infection rate of GLS 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 and 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.

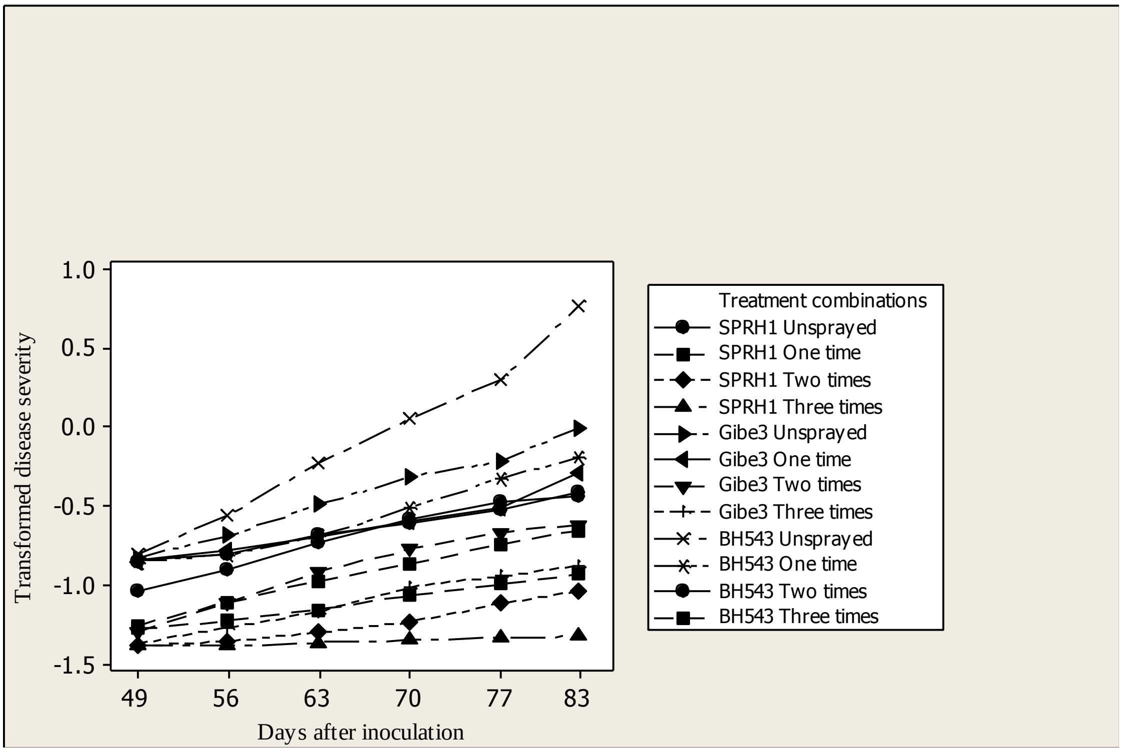

Figure 2. Disease progress curve on maize varieties and their interactions with propiconazole spray frequencies at Bako in 2019/20 main cropping season.

3.1.5. Interaction Effects of Maize Varieties and Fungicide Spray Frequencies on Grain Yield

The integration of varieties with fungicide spray frequencies resulted in a significant ($p<0.05$) difference in mean grain yield. When compared to unsprayed plots of SPRH1, Gibe and BH543 varieties, three times application of fungicide raised the yield from 5.50 to 8.33t, 4.50 to 8.00t and 3.93 to 8.33t respectively (Table 3). The finding is consistent with those of Munkvold et al. (2001), who found that two times application yielded higher yields than single application propiconazole and unsprayed. The genetic potential for yield, disease resistance, and the effect of propiconazole were all factors in the variation in mean grain yield between the hybrid maize varieties studied. Plants often mobilize nutrients and redirect metabolism to support active defense systems under stressful conditions, according to Smith and White (1988), to the detriment of growth and eventual produce. However, by destroying the inoculum prior to infection and/or attempted tissue colonization, propiconazole may have prevented the activation of active defense systems. This would allow more metabolic resources to be allocated to the sink organ, resulting in a higher yield. Petit *et al.,* (2012) also mentioned that triazole fungicides (such as propiconazole) have been shown to improve photosynthesis by increasing chlorophyll content. Furthermore, in a maize crop, thrice spraying with propiconazole resulted in a much higher yield (by 443%) than the control (Ali *et al.,* 2015). From the 2003-2004 cropping seasons in Bako and its surrounding areas or nearby places, yield losses owing to grey leaf spot on resistant, moderately resistant, and susceptible varieties varied from 0-14.9, 13.7-18.3, and 20.8-36.9%, respectively (Wegari et al., 2004).

3.1.6. Relative Yield Loss (RYL)

Estimates of RYL for maize varieties were derived from the treatments that provided the best protection and yield. To compute RYL, all tested (BH543, Gibe 3, and SPRH1) varieties were compared to the maximal protected treatment. Three times application of fungicide reduced the RYL from 52.82% to 0% on BH543 variety. Likewise, on Gibe3 and SPRH1 Variety the maximum protected, reduced from 43.75% to 0% and 33.97% respectively (Table 4). The obtained findings support the effectiveness of combining propiconazole frequencies with maize varieties in minimizing GLS-related adverse effects or epidemics. The findings of this study correspond with those of

Stromberg (2009), who found that higher disease pressure reduced grain yield, which was mostly due to increased blighting and premature death of photosynthetic tissues prior to grain filling. Similarly, Mengist and Moges (2019) found that local maize (check) had the largest yield loss (47.25 %) in maize varieties grown in the field, followed by AMR-852 maize variety with 47.25 % grain yield losses.

Table 5. Integrated effects of hybrid varieties and propiconazole spray frequencies on grain yield and relative yield loss at Bako during the 2019/20 cropping season.

Varieties	Propiconazole Frequencies	Grain yield(t ha^{-1})	Relative yield loss (%)
SPRH1	Unsprayed	5.50^{c}	33.97
SPRH1	One time	8.04^{a}	3.48
SPRH1	Two times	8.17^{a}	1.92
SPRH1	Three times	8.33^{a}	0
Gibe3	Unsprayed	4.50^{e}	43.75
Gibe3	One time	5.25^{cd}	34.38
Gibe3	Two times	6.50^{b}	18.75
Gibe3	Three times	8.00^{a}	0
BH543	Unsprayed	3.93^{f}	52.82
BH543	One time	5.00^{d}	39.98
BH543	Two times	6.80^{b}	18.37
BH543	Three times	8.33^{a}	0
LSD		0.40	
CV		3.57	

Mean values with the same letter within the column are not significantly different at described probability level, LSD=least significant difference, CV=coefficient of variation

3.1.7. Association of Disease Parameters with Yield and Relative Yield Loss

Disease parameters (DI, PSI, AUDPC, and progress rate) were negatively correlated with grain yield but, positively correlated with RYL. Disease severity had a negative correlation with grain yield (r=-0.12), according to Tillahun et al. (2012). Similarly, Nega et al. (2018) discovered a negative association between initial PSI and grain yield (r=-0.08). Despite the fact that the substantial correlation was dependent on the maize varieties and disease parameters, this result is consistent with the findings of the previous two authors. On the susceptible hybrid variety BH543, the majority of disease parameters were substantially (negatively or positively) associated with grain yield and RYL. On the other two maize varieties, comparable findings were observed (Table 4).

Table 6. Association of disease parameters with yield and relative yield loss at Bako in 2019/20 main cropping season.

Disease parameters	Yield (t ha^{-1})	Relative yield loss
	BH543	
Incidence	-0.81**	0.80**
PSI	-0.91**	0.93**
AUDPC(%-days)	-0.91**	0.92**
Progress rate (Unit day^{-1})	-0.75*	0.77**
	Gibe3	
Incidence	-0.82**	0.83**
PSI	-0.98**	0.98**
AUDPC(%-days)	-0.98**	0.98**
Progress rate (Unit day^{-1})	-0.58*	0.58*
	SPRH	
Incidence	-0.70*	0.27NS
PSI	-0.96**	0.19NS
AUDPC(%-days)	-0.96**	0.19NS*
Progress rate (Unit day^{-1})	-0.59*	0.33NS

AUDPC=area under disease progress curve, PSI= percent severity index, Ns=non-significant, *=significant ($p<0.05$), **=significant ($p<0.01$)

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3.1.8. Cost/Benefit Analysis

When compared to untreated controls, integrated maize GLS management resulted in lower disease levels, higher maize grain yield, gross revenue, marginal benefit, and marginal rate of return (MRR). Except for the SPRH1 maize variety, all varieties treated with three sprays of propiconazole yielded the highest marginal benefit. Three sprays of the fungicide on the SPRH variety yielded a lower marginal benefit than one and two sprays on the same variety.Thus, a single spray of propiconazole on the SPRH1 maize variety yielded the highest marginal benefit (ETB 60486), followed by SPRH1 treated twice, SPRH treated three times, and BH543 treated three times, with ETB of 60460.5, 60435, and 60435 respectively. The unsprayed hybrid variety of BH543 provided the lowest marginal benefit (ETB 30,064.5 ha^{-1}).Similarly, the maize variety of SPRH that received one spray of this fungicide had the highest marginal rate of return (1805%), followed by BH543 (992.5%) hybrid sprayed three times with propiconazole (Table 5). This means that for every ETB 1.00 spent on propiconazole and a one-time spray, ETB 18.05 was gained on the SPRH maize variety and ETB 9.93 was gained on the BH543 variety that was treated three times. As a result, maize hybrid SPRH1 sprayed once in one cropping season yielded the highest net profit, marginal benefit, and marginal rate of return. Furthermore, unlike all other varieties, this hybrid maize variety is storage pest resistant (SPR), meaning it resists weevil in storage. So, when the fungicide is sprayed once in a cropping season, production of the SPRH1 maize hybrid variety under propiconazole-sprayed techniques is highly profitable.

Table 7. Cost/benefit assessment of propiconazole fungicide application frequencies against GLS on three hybrid maize varieties at Bako in 2019/20 main cropping season.

Fungicide frequencies	Varieties	Yield (t ha^{-1})	Adjusted yield (%)	MSP (ETkg^{-1})	SR (ETB ha^{-1})	TIC (ETB ha^{-1})	MC (ETB ha^{-1})	NP(ETB ha^{-1})	MB(ETB ha^{-1})	MRR (%)
Unsprayed	BH543	3.93	3537	8.5	30064.5	5250	0	24814.5	30064.5	0
	Gibe3	4.50	4050	8.5	34425	5250	0	29175	34425	0
	SPRH1	5.50	4950	8.5	42075	5250	0	36825	42075	0
One time	BH543	5.00	4500	8.5	38250	6270	1020	31980	37230	702.5
	Gibe3	5.25	4725	8.5	40162.5	6270	1020	33892.5	39142.5	462.5
	SPRH1	8.04	7236	8.5	61506	6270	1020	55236	60486	1805
Two times	BH543	6.80	6120	8.5	52020	7290	2040	44730	49980	976.25
	Gibe3	6.50	5850	8.5	49725	7290	2040	42435	47685	650
	SPRH1	8.17	7353	8.5	62500.5	7290	2040	55210.5	60460.5	901.25
Three times	BH543	8.30	7470	8.5	63495	8310	3060	55185	60435	992.5
	Gibe3	8.00	7200	8.5	61200	8310	3060	52890	58140	775
	SPRH1	8.30	7470	8.5	63495	8310	3060	55185	60435	600

MSP=maize selling price, SR = Sale revenue, TIC = Total input cost, MC = Marginal cost, NP = Net profit. MB = Marginal benefit, MRR = marginal rate of return**.**

Conclusions

Integration of maize varieties and propiconazole spray frequencies had promising effect in reducing grey leaf spot epidemics and increasing grain yield. The highest marginal benefit and marginal rate of return were obtained from variety SPRH1 with once application of propiconazole. Hence, from the economic point of view, instead of using several level fungicides indiscriminately, it is recommended to use SPRH1 variety by one-time application of propiconazole. Thus, economic analysis revealed that the combination of the variety by one-time propiconazole spray frequency could give maximum net benefit and minimize cost of production. To confirm whether the findings from the study areas would be repeated and sustained in several seasons, additional research in other agro-ecologies should be conducted. Host resistance integrated with other cultural practices applicable in the area should be also given due attention to provide other alternatives for effective, efficient and sustainable GLS management options. But, identifying fungicides that can be integrated with other options and are both safe and efficient should be done in the future.

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