Short Communication

Characterization of Temporal Attribute of Turcicum Leaf Blight Epidemics of Maize at Ambo and Bako Districts

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Abstract

Turcicum Leaf Blight (TLB) of maize which is caused by Exserohilum turcicum (Pass.) Leonard and Suggs is a major foliar disease in Ethiopia causing yield loss in the range of 13.6 to 56% depending upon the genotype. Field Experiments were conducted for two consecutive years in two locations viz. Ambo and Bako Agriculture Research Centers to study the temporal attributes of the disease. Six parental lines which constituted tolerant (line1), susceptible (line2), resistant (line5) and three moderately resistant lines (lines 3, 4 and 6) selected from highland maize screening tests were used for the experiments. A randomized complete block design with three replications was used. In 2014, at Ambo, the AUDPC and severity of lines 1, 2 and 3 significantly varied from lines 4, 5 and 6 ($P = 0.0008$ and $P = 0.0005$, respectively). The apparent infection rate was not significant among all the lines in the same year. However, there were no significant variations in the AUDPC, severity and apparent infection rate of TLB among the six lines in 2015. These parameters did not significantly vary in 2014 at Bako. TLB disease was explained by the Logistic model on maize lines 1and 4 in 2014 at Ambo while it was fitted by the Gompertz model on maize lines 2 and 3.TLB disease also explained by the monomolecular disease progress model on lines 5 and 6. None of the models was able to effectively explain TLB disease progress at Bako in 2014 and at Ambo in 2015. Additional studies involving more maize lines and locations are recommended to adequately explain the epiphytotic of the disease and to recommend resistant lines for breeding programs.

Keywords: Maize, Turcicum Leaf Blight, Exserohilum turcicum, temporal attributes, disease progress models

Introduction

Maize (*Zea mays* L.) is one of the popular crops grown in the world, ranking second to wheat and followed by rice (Vasal, 2000). It occupies an important position in the world economy as food, feed, and industrial grain crop. It is a staple food for several million people in the developing world where they derive their protein and calorie requirements from it.

Maize is among the leading cereal crops selected to achieve food self-sufficiency in Ethiopia (Bello *et al*., 2010). Although, improved cultivars have been largely included in the national extension package, the national average yield of maize is only 3.45 tons/ha (CSA, 2015), which is far below the world average of 5.5 tons/ha.

The low yield is attributed to a number of factors such as Biotic (Diseases, insect pests, and weeds), abiotic (moisture, soil fertility, etc). Among biotic factors, foliar diseases such as turcicum leaf blight (*Exserohilum trurcicum*)and common rust (*Puccinia sorghi* Schw) are generally among the important constraints in tropical maize production (Renfro and Ullstrup, 1996).

Most of the composites and hybrids, which are being cultivated commercially susceptible to TLB. In Ethiopia, TLB in maize can cause yield loss in the range of 13.6 to 56.0 per cent depending upon the genotype (Unpublished data). To generate TLB disease epidemics under economic threshold level some works have been done in south Africa by studying spatial attribute of TLB disease and basic information's have been generated to design how to tackle TLB disease in space. Plant disease epidemics can also be described by analyzing disease spread over time (Campbell and Madden, 1990). Such analysis often referred to as temporal studies. Several disease progress models have been proposed for characterizing increase in disease over time for polycyclic diseases with the logistic and Gompertz models being most frequently used (Campbell and Madden, 1990). These models define disease progress in terms of rate of disease increase and estimated disease level at the observed start of the epidemic. Such study provide basic information to device TLB management tactics, thus in Ethiopia where maize is the back bone of the country generation of such basic information is very crucial. Therefore, the objective of this work was to study on the temporal attributes of Turcicum leaf blight epidemics of maize under two agro-ecological zones.

Materials and Methods

Experiment was done at Ambo and Bako Agriculture Research Center for two consecutive years (2014 – 2015 main cropping seasons). Ambo Plant Protection Research Center (APPRC) is located at 08^0 96' 885" N latitude and 37^0 85' 923" E longitude and at an altitude of 2147m.as.l. The annual average temperature and rain fall is 27.54^0C and 1077.68 mm, respectively. Bako is located at an altitude of 1650 m.a.s.l, 9°06′ north latitude and 37°09′ east longitude. Average annual rainfall at this location is 1246 mm.

Six parental lines were selected from screening test made at ambo and Bako in the previous cropping season. These were constituted susceptible, Tolerant, moderately resistant and resistant maize lines. At both locations, the plots were tractor ploughed and disc harrowed twice before planting. The plots were arranged following a randomized complete block design (RCBD) with three replications. The experimental unit measured 4.5 x 4.5m with 6 maize rows planted at a spacing of 75 X 30 cm. All plots were planted by hand with two seeds per hole. Inorganic fertilizer (Dap &Urea) and all agronomic practices were applied based on the area recommendations.

Sample collection, Culturing, mass multiplication, Inoculation and disease assessments

Disease samples were collected from infected maize fields of APPRC and isolated by culturing on the potato dextrose agar (PDA) using the following standard tissue isolation technique as mentioned below.

The necrotized leaf bits along with some healthy portions was surface sterilized in 70% alcohol solution for 30 seconds and washed thoroughly thrice in sterile distilled water. Then such bits were aseptically transferred to sterile potato dextrose agar (PDA) Petridishes. The Petridishes were incubated at 27±1°C and observed periodically for fungal growth. The pure colonies which were developed from the bits transferred to PDA slants and incubated at room temperature for 15 days. After fifteen days when abundant sporulation was occurred; TLB pathogen was purified following hyphal tip isolation technique and kept in refrigerator at 5°C which was used at field inoculation.

One hundred grams of sorghum grains was placed in 500 ml conical flask and soaked in tap water for 24 hours. The material was sterilized twice at 24 hours interval using autoclave. The contents of the flasks were thoroughly shaken after sterilization to prevent clumping. The flasks were aseptically inoculated with *E. turcicum* culture and incubated at 27±1°C for 20 days and they shake every alternate day to avoid clumping. Within three weeks, fully colonized sporulated sorghum grain culture was used for creating artificial epiphytotic conditions at field.

Maize plants in each plot were inoculated by placing approximately equal amount of *Exserohilum turcicum* on maize ears using pinching at 4-5 leaf stages. After inoculation, water was sprayed with hand atomizer to create favorable conditions for pathogen germination. One week after inoculation, plots were assessed for disease severity using a 1 to 5 scale (Payak and Sharma, 1982). Severity scores were converted to percent disease index (PDI) as described by Wheeler (1969) using the formula below;

$$PDI = \frac{Sum\ of\ numerical\ grading}{Plants\ examined\ x\ maximum\ disease\ grade} \text{ X } 100$$

Disease assessment commenced 7 days after inoculation. Six assessments were made at 7 days intervals from four central tag maize plants with visual observations.

Modeling temporal disease spread and data analysis

Turcicum leaf blight symptoms were observed on each test plant by visual assessment on the leaves. Plants were assessed for six weeks. Disease measurements were subjected to ANOVA. Severity data was used to compute areas under disease progress curves (AUDPC), as well as tested to check logistic, monomolecular and Gompertz models. Areas under disease progress curves (AUDPC) were computed according to Campbell and Madden (1990). The formulae for computing AUDPC is given as

$$AUDPC = \sum_{i=1}^{n-1} 0.5(x_{i+1} + x$$

Where, X_i is the cumulative disease severity expressed as a proportion at the i^{th} observation, t_i is the time (days after planting) at the i^{th} observation and n is total number of observation.

Apparent infection rate is an estimate of the rate of progress of a disease, based on proportional measures of the extent of infection at different times.

Firstly, a proportional measure of the extent of infection was chosen as the disease extent. For example, this might be the proportion of leaf area affected by mildew, or the proportion of plants in a population showing dieback lesions. Measures of disease extent are then taken over time, and a mathematical model is fit. The model was based upon two assumptions:

- the progress of the infection is constrained by the amount of tissue that remains to be infected; and
- if it were not so constrained, the extent of infection would exhibit exponential growth.

There is a single model parameter *r*, which is the apparent infection rate. It can be calculated analytically using the formula

$$r = \frac{1}{t_2 - t_1} \log_e \left[\frac{x_2(1 - x_1)}{x_1(1 - x_2)}\right]$$

Where: *r* is the apparent infection rate, t_1 is the time of the first measurement, t_2 is the time of the second measurement, x_1 is the proportion of infection measured at time t_1. x_2 is the proportion of infection measured at time t_2

Fitting of the data to growth curve models of Logistic and Gompertz were performed to characterize the polycyclic nature of epidemics. The slope of the curve, (r) depicts rate of disease increase over time, and y, the theoretical estimates of initial amount of epidemic y_0 (y-axis intercept). The Logistic model was given by

$$\mathrm{Ln}\,(y/_{(1-y)}) = \ln\,(y_0/_{(1-y0)}) + rt$$

Computation of y and r (apparent rate of infection) was performed over time. Data was also fitted to the Gompertz model (Berger, 1981) as described for the logistic models expect that the linearized formula for the Gompertz model may be different, i.e. it is given by

$$-\ln\{-\ln(y)\} = -\ln\{(-\ln y_0)\} + rt$$

These models are selected because of their common usage and suitability for different fungal diseases. In these models y is proportion of disease severity at time (t) due to inoculum application and background infection and r is the slope. The appropriateness of each model was evaluated on the basis of coefficient of correlation (R^2) and mean square error (MSE). Models were selected which had high values of R^2 and low values of MSE.

As appropriate, data was subjected to ANOVA and if significant differences were found, means were compared using Fisher's Protected Least Significant Differences (LSD) at $P \leq 0.05$.

Results and Discussion

There was statically variation among maize lines in mean area under disease progress curve and mean severity of Turcicum leaf blight (Table 1). Maize lines of [KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2)-32-2-2-1-1-#-# (line 1), 142-1-eQ (line 2), and [KIT/SNSYN[N3/TUX]]c1F1-##(GLS=1)-21-2-3-1-1-1-# (line 3) showed the highest mean area under disease progress curve (AUDPC) and mean severity at Ambo in 2014 even though statically at par. The lower AUDPC and severity was recorded on the 3 remaining maize lines and they did not show statically variation. However, there was no statically variation among six maize lines in apparent infection rate of TLB at Ambo in 2014 (Table 1)

Table 1. AUDPC. Apparent infection rate and severity of TLB on six maize lines at Ambo in 2014

Line	AUDPC % days	Rate /day	Severity (%)
[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2)-32-2-2-1-1-#-# or **line 1**	**541.03a**	**0.0833**	**37.25a**
142-1-eQ or **line 2**	**516.41a**	**0.2067**	**33.47a**
[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=1)-21-2-3-1-1-1-# or **line 3**	**414.9a**	**0.1533**	**27.77a**
[POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-1-#-#-#-# or **line 4**	**263.73b**	**0.2667**	**17.86b**
[POOL9Ac7-SR(BC2)]FS67-1-2-3-1-#-#-#-#-# or **line 5**	**213.5b**	**0.2967**	**13.80b**
[POOL9Ac7-SR(BC2)]FS89-1-2-4-2-1-1-1-#-#-# or **line 6**	**200.07b**	**0.2733**	**14.51b**
P=	**0.0008**	**0.75**	**0.0005**
LSD=	**144.02**	**ns**	**6.11**
CV=	**22.1**	**92.47**	**11.64**

There were no statically significant differences among six maize lines in AUDPC, apparent infection rate and severity of Turcicum leaf blight (TLB) at Bako in 2014 (Table 2). **Similarly,** there were no statically significant differences among six maize lines in AUDPC, apparent infection rate and severity of Turcicum leaf blight at Ambo in 2015 (Table 3). But the highest AUDPC of TLB was recorded at Ambo in 2015 from six maize varieties.

Table 2. AUDPC, Apparent infection rate and severity of TLB on six maize lines at Bako in 2014

Line	AUDPC % days	Rate /day	Severity (%)
[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2)-32-2-2-1-1-#-# or **line 1**	398.2	0.0667	27.76
142-1-eQ or **line 2**	302.4	0.0933	22.57
[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=1)-21-2-3-1-1-1-# or **line 3**	237.4	0.2133	16.89
[POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-1-#-#-#-# or **line 4**	394.5	0.1133	28.56
[POOL9Ac7-SR(BC2)]FS67-1-2-3-1-#-#-#-#-# or **line 5**	418.6	0.0333	31.41
[POOL9Ac7-SR(BC2)]FS89-1-2-4-2-1-1-1-#-#-# or **line 6**	331.8	0.0867	24.77
P=	0.77	0.59	0.74
LSD=	ns	ns	ns
CV=	49.03	120.52	27.16

Table 3. AUDPC, Apparent infection rate and severity of TLB on six maize lines at Ambo in 2015

Line	AUDPC % days	Rate /day	Severity (%)
[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2)-32-2-2-1-1-#-# or line 1	925.3	0.0263	23.99
142-1-eQ or **line 2**	**990.2**	**0.0183**	**25.69**
[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=1)-21-2-3-1-1-1-# or **line 3**	**1195.2**	**0.0233**	**32.06**
[POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-1-#-#-#-# or **line 4**	**1080.1**	**0.0470**	**29.43**
[POOL9Ac7-SR(BC2)]FS67-1-2-3-1-#-#-#-#-# or **line 5**	**605.2**	**0.0500**	**16.50**
[POOL9Ac7-SR(BC2)]FS89-1-2-4-2-1-1-1-#-#-# or **line 6**	**987.7**	**0.0240**	**26.10**
P=	**0.69**	**0.6500**	**0.74**
LSD=	**ns**	**ns**	**ns**
CV=	**45.52**	**90.1700**	**27.16**

Turcicum leaf blight (TLB) fitted to Logistic model on [KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2)-32-2-2-1-1-#-# (maize **line 1**) and [POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-1-#-#-#-# (maize **line 4**) at Ambo in 2014 (Table 4).

TLB also fitted to Gompertz on maize **line 2** (142-1-eQ) and **line 3** ([KIT/SNSYN[N3/TUX]]c1F1-##(GLS=1)-21-2-3-1-1-1-#); and Monomolecular on maize **line 5** ([POOL9Ac7-SR(BC2)]FS67-1-2-3-1-#-#-#-#-#) **and line 6** ([POOL9Ac7-SR(BC2)]FS89-1-2-4-2-1-1-1-#-#-#) models, respectively. Whereas, Turcicum leaf blight disease was not explained by any models on the six maize varieties at Ambo, in 2015 (Table 6).

TLB was fitted to Monomolecular and Logistic models on maize **line 4** ([POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-1-#-#-#-#) and **line 6** ([POOL9Ac7-SR(BC2)]FS89-1-2-4-2-1-1-1-#-#-#) at Bako in 2014, respectively (Table 5). Whereas, Turcicum leaf blight was not fitted to any model on the remaining 4 maize lines at Bako in that growing season.

Table 4. Model description of TLB on Six maize lines at Ambo in 2014

Line	Model	R-square	MSE	Intercept	Standard error of intercept	Rate (slope)	Standard error of slope
1	**L**	**0.62**	**0.43**	**-1.76**	**0.38**	**0.09**	**0.03**
1	M	0.55	0.16	0.1	0.14	0.03	0.01
1	G	0.6	0.27	-0.7	0.24	0.05	0.02
2	L	0.6	1.09	-4.00	0.96	0.21	0.63
2	M	0.62	0.21	-0.13	0.19	0.04	0.01
2	**G**	**0.65**	**0.47**	**-1.54**	**0.41**	**0.1**	**0.03**
3	L	0.51	0.98	-3.41	0.86	0.15	0.06
3	M	0.46	0.2	-0.04	0.18	0.03	0.01
3	**G**	**0.52**	**0.44**	**-1.31**	**0.39**	**0.07**	**0.03**
4	**L**	**0.51**	**0.63**	**-3.05**	**0.65**	**0.1**	**0.01**
4	M	0.5	0.12	-0.06	0.11	0.02	0.02
4	G	0.48	0.3	-1.19	0.31	0.05	0.03
5	L	0.78	0.52	-4.24	0.53	0.15	0.03
5	**M**	**0.89**	**0.04**	**-0.09**	**0.04**	**0.02**	**0.002**
5	G	0.83	0.17	-1.56	0.17	0.06	0.01
6	L	0.78	0.52	-4.24	0.53	0.15	0.03
6	**M**	**0.89**	**0.04**	**-0.09**	**0.04**	**0.02**	**0.002**
6	G	0.83	0.17	-1.56	0.17	0.06	0.01

Table 5. Model description of TLB on Six maize lines at Bako in 2014

Line	Model	R-square	MSE	Intercept	Standard error of intercept	Rate (slope)	Standard error of slope
1	L	0.2	0.84	-2.03	0.74	0.07	0.05
1	M	0.17	0.23	0.14	0.19	0.02	0.013
1	G	0.19	0.45	-.075	0.4	0.03	0.03
2	L	0.17	0.76	-2.14	0.67	0.05	0.04
2	M	0.14	0.17	0.12	0.15	0.01	0.01
2	G	0.16	0.39	-0.8	0.35	0.03	0.02
3	L	0.39	0.51	-2.58	0.45	0.06	0.03
3	M	0.31	0.12	0.03	0.1	0.01	0.006
3	G	0.36	0.26	-1.02	0.23	0.03	0.02
4	L	0.82	0.35	-2.67	0.31	0.12	0.02
4	**M**	**0.83**	**0.09**	**-0.09**	**0.08**	**0.03**	**0.005**
4	G	0.83	0.19	-1.16	0.17	0.06	0.01
5	L	0.05	0.89	-1.35	0.79	0.03	0.05
5	M	0.05	0.25	0.28	0.22	0.008	0.015
5	G	0.05	0.49	-0.41	0.44	0.02	0.03
6	**L**	**0.54**	**0.67**	**-2.83**	**0.59**	**0.11**	**0.04**
6	M	0.48	0.22	-0.14	0.19	0.03	0.01
6	G	0.51	0.4	-1.24	0.35	0.06	0.02

L = Logistic; M = Monomolecular; G = Gompertz

Table 6. Model description of TLB on Six maize lines at Ambo in 2015

Line	Model	R-square	MSE	Intercept	Standard error of intercept	Rate (slope)	Standard error of slope
1	L	0.05	0.86	-1.62	0.52	0.02	0.02
1	M	0.03	0.24	0.23	0.15	0.004	0.006
1	G	0.04	0.48	-.054	0.29	0.009	0.01
2	L	0.17	0.56	-1.31	0.34	0.02	0.01
2	M	0.24	0.17	0.21	0.11	0.009	0.005
2	G	0.2	0.33	-0.46	0.2	0.02	0.009
3	L	0.17	0.56	-1.31	0.34	0.02	0.01
3	M	0.24	0.17	0.21	0.11	0.009	0.005
3	G	0.2	0.33	-0.46	0.2	0.02	0.009
4	L	0.17	0.61	-1.49	0.37	0.03	0.02
4	M	0.17	0.17	0.21	0.1	0.007	0.005
4	G	0.17	0.34	-0.52	0.21	0.01	0.009
5	L	0.48	0.4	-2.47	0.24	0.04	0.01
5	M	0.41	0.1	0.02	0.06	0.008	0.003
5	G	0.45	0.21	-0.99	0.13	0.02	0.006
6	L	0.12	0.66	-1.62	0.4	0.02	0.02
6	M	0.19	0.19	0.14	0.12	0.009	0.005
6	G	0.15	0.37	-0.62	0.23	0.01	0.009

Summary and Recommendation

Turcicum leaf blight (TLB) is among devastating foliar fungal diseases of maize in Ethiopia. Temporal epidemiology was studied at two agro-ecological zones of the country using six maize lines. There was significant difference among the six lines in AUDPC and severity while apparent infection rates of TLB did not differ in 2014 at Ambo. These parameters did not significantly vary in 2014 at Bako and in 2015 at Ambo.

TLB was fitted to Logistic, Gompertz and Monomolecular models on different maize lines at Ambo in 2014 growing season. Except on maize **line 4** and **6**, none of the models was able to effectively explain TLB disease at Bako in 2014. Similarly, TLB disease was not fitted to any models on six maize lines at Ambo in 2015 growing season.

Therefore, additional studies involving more maize lines and locations are recommended to adequately explain epiphytotic of TLB disease and to recommend resistant lines for breeding programs. Race analysis should be done in order to check race variability. Moreover, disease intensities should be correlated with environmental factors.

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References

Bello OB, Abdulmalig SY, Afolabi MS, Lge SA. 2010. Correlation and path coefficient hybrids analysis of yield and agronomic characters among open pollinated maize varieties and their F1in a diallel cross. Afr.J.Biotech. 9(18): 2633-2639.

Payak MM, Sharma RC. 1982. Maize diseases and approaches to their management in India. *Trop Pest Management* 31: 302-310.

Campbell, C.L. and Madden. L.M.1990. *Introduction to Plant Disease Epidemiology*Newyork: John Wiley and Sons

CSA (Central Sastical Authority). 2015. Agriculture sample survey report on area and production for major crops for 2014/015. The FDRE Sastical Buletin. Addis Ababa, Ethiopia.

Renfro. B.L. and Ullstrup, AJ.1996. Comparison of maize disease in temperate and tropical environments.PANS 22:491-498.

Vasal, S.K. 2000. Quality maize story. In: Improving Human Nutrition Through Agriculture. The Roll of International Agricultural Research. A workshop hosted by IRRI, Philippines, Organized by International Food Policy Institute. Los Banos. October 5-7, 1999.

Wheeler, B.E.J. 1969. An introduction to plant diseases. John Wiley and Sons LTD, London.