



# Effect of different potassium rates on the severity of Northern Corn Leaf Blight caused by *Helminthosporium turcicum* in North Benin

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## ABSTRACT

**Objective:** The overall objective is to develop a method to control the Northern Corn Leaf Blight caused by *Helminthosporium turcicum*, one of the major constraints in the worldwide maize production and particularly in Benin. Specifically, it aims to determine the optimal potassium rate to act effectively on the disease.

**Methodology and Results:** The study consist of subjecting three maize varieties to the effect of four different rates of K<sub>2</sub>O (40, 48, 60 and 90 kg K<sub>2</sub>O/ha abbreviated as K<sub>40</sub>, K<sub>48</sub>, K<sub>60</sub> and K<sub>90</sub>) in the field at Gogounou (North Benin) in 2015 and 2016 then in the greenhouse in 2016. The experimental design was a split plot with four repetitions with K fertilizer rate as main factor, and variety as secondary factor. The study revealed that the rates K<sub>48</sub>, K<sub>60</sub> and K<sub>90</sub> respectively reduce the severity of Northern Corn Leaf Blight by 24.17%, 40.46% and 48.80% compared to the rate K<sub>40</sub> in the greenhouse. In the field, the rates K<sub>60</sub> and K<sub>90</sub> respectively reduce the severity by 32.56% and 8.87% compared to the rate K<sub>40</sub> in 2015. In 2016, the rates K<sub>48</sub>, K<sub>60</sub> and K<sub>90</sub> reduce the severity respectively by 9.83%, 23.96% and 15.39% compared to the K<sub>40</sub> rate. In addition, no significant difference was found between the severity values of the three varieties studied neither in the greenhouse nor in the field. In addition, no significant correlation was observed between severity and grain yield in the field during the two years.

**Conclusions and application of findings:** The application of potassium decreases the severity of Northern Corn Leaf Blight and the rate K<sub>60</sub> is the optimal to reduce the disease. The availability of fertilizer at the K<sub>60</sub> to farmers in the endemic zones could help for sustainable management of the disease.

**Keywords:** *Helminthosporium turcicum*, maize, Northern Corn Leaf Blight, North-Benin, potassium.

## INTRODUCTION

In West Africa, maize (*Zea mays* L.) is the only cereal crop with remarkable production potential with a better yield than other cereals; and is the staple food of about 50% of the population (Badu & Fakorede, 2017). In Benin, maize is the main cereal

grown and represents 84% of cereal production (Soule et al., 2008). An in-depth analysis of the dynamics of maize production in Benin over the past fifteen years reveals that it ranged from around 788,320 tons in 2003 to 1,509,758 tons in 2018

(FAOSTAT, 2020). In addition, maize yields in real environments stagnate between 1.1 to 1.50 t/ha compared to 4 to 5 t/ha in semi controlled conditions (Badu & Fakorede, 2017). Among the biotic factors that limit the production and productivity of maize are fungal diseases including northern corn leaf blight. In Benin, considerable yield losses of maize can be caused by fungal leaf attacks (Sikirou, 2014). Northern corn leaf blight, caused by a pathogenic ascomycete in cereals, *Helminthosporium turcicum* (syn. *Exserohilum turcicum* (Pass.) K. J. Leonard & Suggs), is a major problem in maize production worldwide (Asea et al., 2011; Zhu et al., 2011; Technow et al., 2013). Symptoms of the disease occurs in the form of small, moist oval spots which progress and join together to form elongated spindle-shaped necrotic lesions (Paliwal et al., 2002), usually 12 mm wide, 3- 15 cm long with yellow - gray at the centers and red at the edges (King & Mukuru, 1994) (Fig 1A). In case of heavy infestation, the leaves can dry prematurely and completely like in plants suffering from drought (Paliwal et al., 2002). This disease alone is capable, under favourable conditions and in early infection, of causing yield losses estimated at 70% (Santiago et al., 2013) or even 91% (Pant et

al., 2001; Singh et al., 2012; Ishfaq et al., 2014; Nwanosike et al., 2015). In addition to maize, this disease attacks sorghum, resulting in yield losses of up to 70% on susceptible cultivars (Ramathani, 2010; Yeshitila, 2003). The disease management requires the combination of agronomic and genetic or even chemical measures (AGPM, 2014). One of these strategies could be good management of crop fertilization because according to Anderson (2002), there is an interaction between nutrients and plant diseases. Indeed, according to Geary & Jacobson (2016), balanced plant nutrition is an effective and affordable way to minimize or prevent plant diseases. Sanogo & Yang (2001) have shown that potassium nutrition has an effect on sudden soybean death syndrome and that the application of potassium chloride promotes an average reduction of 36% in the severity of the disease. In Nepal and Morocco, Sharma et al. (2005) and Imrani et al. (2014) have also shown that the application of potassium decreases the severity of wheat and rice leaf blight, respectively. The present study therefore aims to manage *Helminthosporium turcicum* infection through the application of an effective rate of potassium.

## MATERIALS AND METHODS

**Plant material:** it consisted of three (03) improved varieties of healthy maize but susceptible to northern corn leaf blight and supplied by the Center for Agricultural Research of Ina (CRA-Ina). These are two (02) early varieties (DMRESR-W and EVDT-97-STR) and one (01) intermediate (FAABA / QPM9).

**Experimental sites:** The field trials were installed in the commune of Gogounou (10°50'.391"N and 002°49'.460"E) during the periods from July to November 2015 and from June to November 2016. The total rainfall was 986 mm in 2015 and 943 mm in 2016. The humidity was between 61 and 90% in 2015 and between 69 and 94% in 2016. The temperature varied from 21.4 to 31.8°C in 2015 and from 20.9 to 28.3°C in 2016. The greenhouse trial was conducted at the Faculty of Agronomy (University of Parakou in North Benin) in August 2016. The climate is Soudan-Guinean type and characterized by a rainy season from April to October and a dry season from November to March (Afrique

Conseil, 2006a, b). The soils are those of the granitogneissic base, mostly ferruginous.

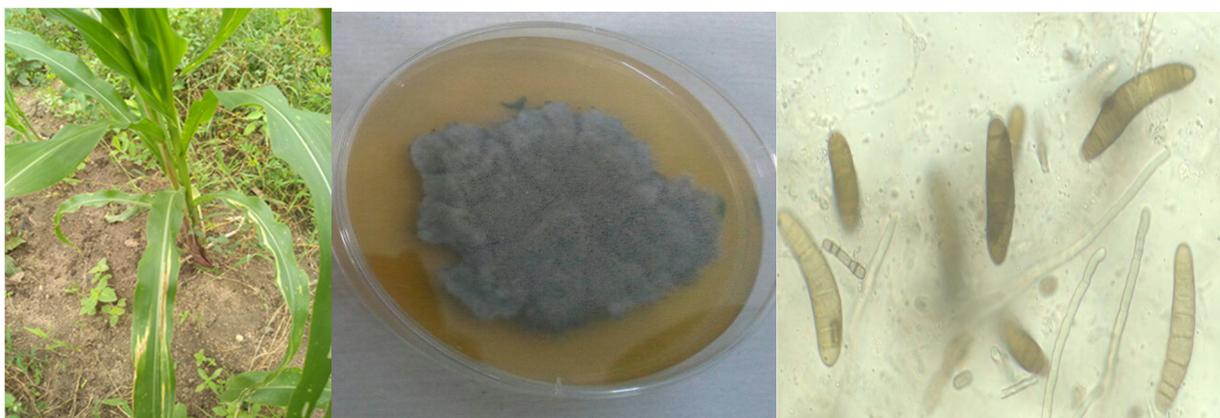
**Experimental design:** For the field experiments, a split plot design with four replicates was used with potassium rates as the main factor and variety as the secondary. Each block was subdivided into four (04) sub-blocks. Each sub-block was composed of three (03) elementary plots of 10 m<sup>2</sup> (5 m x 2 m) on which the three (03) varieties were sown (two early varieties (DMRESR-W and EVDT-97-STR) and an intermediate (FAABA/QPM9)] subject to the same fertilizer rates. The four potassium rates were: i) 40 Kg K<sub>2</sub>O /ha (K<sub>40</sub>) as a control (Farmer's practice); ii) 48 Kg K<sub>2</sub>O /ha (K<sub>48</sub>), iii) 60 Kg K<sub>2</sub>O /ha (K<sub>60</sub>) and iv) 90 Kg K<sub>2</sub>O /ha (K<sub>90</sub>). N and P were applied to all plots at the recommended rate of 60 kg N ha<sup>-1</sup> and 40 kg P<sub>2</sub>O<sub>5</sub>/ha in the study area. N was applied as urea (60% N), P as triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>) and K as muriate of potash (60% K<sub>2</sub>O) at 21 days after sowing. Two seeds were sown manually per hole, taking as spacing 80 cm between rows and 40 cm

between holes, giving a maximum density of 62,500 plants per hectare. Note that two successive blocks were 2m apart while the sub-blocks were separated by aisles of 1m, in the same way as the elementary plots. Additional urea was applied forty-five days after sowing at a dose of 50kg/ha, i.e. an amount of 3.48 g of (N) per hole. The maintenance of the plots consisted of making two manual weeding during the whole maize cultivation cycle. Thus, no phytosanitary treatment has been carried out. For the greenhouse experiment, the same design was implemented in August 2016. An elementary plot was represented by 2 plastic pots of 1.5 dm<sup>3</sup> each, with two plants per pot. The potting soil used was sterilized at 65°C for 72 hours. The temperature in the greenhouse ranged from 20.7 to 26.7°C and the relative humidity between 76 and 96%.

**Soil sampling and analysis:** In 2015, before sowing, a composite soil sample (0.2 m deep) was taken from several randomly selected locations throughout the experimental field. This sample reflects the condition of the soil before application of the treatment. All visible

organic residues were removed by hand, and then all samples from each plot were thoroughly mixed to obtain a composite sample for analysis. The sample was analysed according to the methods of Tran & Boko (1978) cited by Kate et al. (2016), at the "Laboratoire des Sciences du Sol, Eaux et Environnement (LSSEE/CRA-Agonkanmey / INRAB)".

**Isolation and inoculation of the *H. turcicum*:** Two virulent *H. turcicum* isolates UP-GoS3 and UP-PkS2 obtained from previous surveys in Gogounou and in Parakou (North Benin) were prepared by growing them on Potato Dextrose Agar medium (potato extract: 4g/l, dextrose 20g/l, agar 15g/l) at 25°C for 48 hours. Sub-cultures were grown at 25°C for 10 days (Fig. 1). Conidia suspensions were prepared with sterilized distilled water and adjusted to 2 10<sup>4</sup> conidia/ml using a hemacytometer. The prepared inoculum was applied to leaf surfaces with a hand sprayer after adding one drop of Tween 20. Plants sprayed with sterilized distilled water served as the control.



**Figure 1:** Symptoms of *H. turcicum* (A) Ten days culture of *H. turcicum* on PDA (B) and Conidia of *H. turcicum* (1000X) (C)

**Disease and yield assessment:** Two weeks after inoculation, disease index were evaluated on field and greenhouse plants by scoring individual plants. The disease index in the field experiment was assessed on twenty (20) plants chosen at random and marked on each elementary plot using the diagonal method and per elementary plot (2 pots) in the greenhouse experiment. The modified Saari & Prescott (1975) scale from 0 to 5 was adopted to assess this severity across the infected leaf surface, with:

(0 = <1%; 1 = 1-20%; 2 = 21-40 %; 3 = 41-60%; 4 = 61-80% and 5 => 80%).

These assessments were used to calculate the disease severity as follow:

$$S = (\sum n) / (N \times 5) \times 100$$

with **S** = Disease severity, **n** = individual index, **N** = Total number of plant assessed, **5**: High index value.

Four-disease index assessment was performed at two weeks apart. The values from these assessments were used to calculate the disease severity per plot and then the area under severity curve as follow:

$$\text{AUSPC} = \sum_i [(S_i + S_{i-1}) * (t_i - t_{i-1})] / 2$$

with  $t_i$  = date of assessment,  $S_i$  = average of severity at  $t_i$  (Shaner & Finney, 1977)

For the yield assessment, the plants in the central row (4 m<sup>2</sup>) were harvested from each elementary plot to estimate grain yields. The grain moisture content was then determined for each replication after oven drying at 65°C to a constant mass. The number of plants and the number of maize cobs per plant were counted. The dry weight of grains per cob was calculated by dividing the total dry grain weight per replicate by the number of

cobs. Maize grain and stover yields were then calculated and expressed in kg.ha<sup>-1</sup> on a dry weight basis.

**Statistical analysis:** Analysis of variance (ANOVA) was carried out using R version 3.1.2 software on severity (AUSPC) and yield values to compare the means at the 5% threshold and the Tukey test made it possible to separate the means when there was a significant difference ( $p \leq 0.05$ ). The Pearson correlation test ( $p \leq 0.05$ ) was performed between the severity and yield values. The values in the tables are the real means with their standard errors.

## RESULTS

**Soil properties of the field:** The soil is a loamy-sand with approx. 6% clay in the top 0.2 m, acidic with low organic carbon and total nitrogen and medium phosphorus content (Table 1).

**Table 1:** Results of analyses of the field soil sample

Characteristics	Soil contents
Clay (%)	6.262
Stringers (%)	14.378
Sand (%)	79.360
Water pH	6.7
Organic carbon (%)	0.57
Total nitrogen (%)	0.028
C/N	20
Organic material (%)	0.98
Exchangeable Ca <sup>2+</sup> (meq/100g)	1.3229
Exchangeable Mg <sup>2+</sup> (meq/100g)	0.7106
Exchangeable K <sup>+</sup> (meq/100g)	0.1417
Assimilable P (ppm)	6
Sum of cations (meq/100g)	2.290
CEC (meq/100g)	4.320

**Effect of fertilizer rates on the severity of Northern Corn Leaf Blight in the greenhouse:** The severity values of Northern Corn Leaf Blight under greenhouse are significantly different between potassium rates ( $P = 0.001$ ). In fact, the K<sub>40</sub> treatment is the one that least reduces the severity of Northern Corn Leaf Blight under greenhouse (AUSPC = 352.35 ± 27.87) (Table 2). It is followed by rates K<sub>48</sub> and K<sub>60</sub> which moderately reduce the severity of Northern Corn Leaf Blight (AUSPC =

267.16 ± 22.76 and 239.76 ± 14.34, respectively). As for rate K<sub>90</sub>, it is the one that most significantly reduces the severity of Northern Corn Leaf Blight under greenhouse (AUSPC = 180.38 ± 19.39). There is no significant difference between the severity values (AUSPC) of Northern Corn Leaf Blight both at the level of the varieties ( $P = 0.23$ ) nor at the level of the combination of varieties \* rates ( $P = 0.33$ ).

**Table 2:** Severity (AUSPC) of *H. turcicum* leaf blight of 3 maize varieties under 4 potassium rates in greenhouse

Treatments	AUSPC
<u>K rates</u>	
K <sub>40</sub>	352.35±27.78c
K <sub>48</sub>	267.16±22.76b
K <sub>60</sub>	209.76±14.34a
K <sub>90</sub>	180.38±19.39a
Tukey test (0.05)	*
<u>Varieties</u>	
DMRESR-W	271.74±21.95a
EVDT-97-STR	274.1±23.06a
FAABA/QPM9	233.86±19.19a
Tukey test (0.05)	NS
ANOVA K rates * Varieties	NS

NS = no significant difference at  $P \leq 0.05$ ; \* = significant difference at the threshold 5%. ANOVA = analysis of variance.

**Effect of fertilizer rates on the severity of Northern Corn Leaf Blight according to the varieties tested in the field:** No significant difference was noted in 2015 and 2016, respectively, between the values of AUSPC when considering the factors K rates \* varieties ( $P = 0.137$  and  $0.306$ ), then with the variety factor ( $P = 0.446$ ) in 2015. However, in 2016 a significant difference ( $P = 0.024$ ) was noted between the AUSPC values observed at the level of the varieties tested. In addition, there is a significant difference ( $P = 5.9.10^{-6}$  and  $8.10^{-6}$ ) between the AUSPC values observed in the K fertilizer rates in

2015 and 2016 respectively. These AUSPC values were between 72.7 and 135.83 in 2015 then between 446.25 and 586.87 in 2016. This therefore reveals that the four (04) K fertilizer rates tested in 2015 and 2016, act differently with the Northern Corn Leaf Blight infection. Indeed, K<sub>40</sub> and K<sub>48</sub> treatments are the ones that have reduced the least severity of Northern Corn Leaf Blight during the two years. The K<sub>60</sub> treatment was the one that most reduced the severity of Northern Corn Leaf Blight in the field in both 2015 and 2016.

**Table 3:** Severity (AUSPC) of *H. turcicum* leaf blight of 3 maize varieties under 4 potassium fertilizer rates in the fields in 2015 and 2016

Treatments	AUSPC	
	2015	2016
<u>K rates</u>		
K <sub>40</sub>	107.80±9.62bc	586.87±22.97c
K <sub>48</sub>	135.83±9.23c	529.14±18.24bc
K <sub>60</sub>	72.70±11.02a	446.25±19.40a
K <sub>90</sub>	98.23±8.07ab	496.51±17.90ab
Tukey test (0.05)	***	***
<u>Varieties</u>		
DMRESR-W	107.00±10.03a	525.96±17.55ab
EVDT-97-STR	108.79±8.63a	540.51±17.38b
FAABA/QPM9	95.13±5.85a	477.61±16.81a
Tukey test (0.05)	NS	*
ANOVA K rates * Varieties	NS	NS

NS = no significant difference at  $P \leq 0.05$ ; \* and \*\*\* = significant difference at the threshold 5% and 5%, 1%. ANOVA = analysis of variance.

**Grain yield assessment:** No significant difference was noted in 2015 and 2016 between the grain yield values, when we consider the factors: varieties ( $P = 0.274$  and  $0.633$ ) or K rates \* varieties ( $P = 0.128$  and  $0.816$ ) (Table

4). However, with the factor "K rates", a significant difference ( $P = 0.003$ ) was noted in 2015 unlike 2016 ( $P = 0.473$ ).

**Table 4:** Grain yield (t/ha) of 3 maize varieties under 4 potassium fertilizer rates in the fields in 2015 and 2016

Treatments	Grain yields (t/ha)	
	2015	2016
<u>K rates</u>		
K <sub>40</sub>	2.65±0.17a	3.45±0.33a
K <sub>48</sub>	3.18±0.19ab	2.91±0.34a
K <sub>60</sub>	2.62±0.27a	3.54±0.23a
K <sub>90</sub>	3.59±0.18b	3.63±0.40a
Tukey test (0.05)	**	NS
<u>Varieties</u>		
DMRESR-W	3.23±0.19a	3.37±0.26a
EVDT-97-STR	2.94±0.19a	3.60±0.32a
FAABA/QPM9	2.86±0.22a	3.19±0.29a
Tukey test (0.05)	NS	NS
ANOVA K rates* Varieties	NS	NS

NS = no significant difference at  $P \leq 0.05$ ; \*\* = significant difference at the threshold 1%. ANOVA = analysis of variance.

**Correlation severity - grain yield:** According to the results of the correlation analysis, no significant relationship was observed between the severity and the grain yield both in 2015 ( $r = -0.14$  and  $P = 0.353$ ) and in

2016 ( $r = 0, 11$  and  $P = 0.456$ ). The Northern Corn Leaf Blight did not therefore have a particular effect on the grain yields of the different varieties used during our test.

## DISCUSSION

The severity (AUSPC) of Northern Corn Leaf Blight varied according to the fertilizer rates. This result would be because there is a relationship between plant nutrition and phytopathogens. Indeed, according to Bruulsema et al. (2012), the management of plant nutrition has an impact on plant diseases and allows their control. In addition, Anderson (2002) proved that there are interactions between plants, nutrients and pathogenic species by indicating through the results of his work that the severities of leaf spot in rice vary significantly in function of the different K fertilizer rates. These results also corroborate those of Duveiller & Dublin (2000) and Mehta (1993) who reported that an integrated approach including fertilization is the best way to control the *Helminthosporium* leaf blight of wheat. The rate K<sub>90</sub> (having the highest amount of potassium) is the one which has more reduced the severity of the disease in the greenhouse whereas it is the K<sub>60</sub> rate which had the most significant effect both years in the field. This difference in the K rate observed in the greenhouse need to be elucidated through a repeated trial. Indeed, Sharma (2002) proved in Nepal, that the application of potassium decreases the severity of *Helminthosporium* leaf blight in wheat. In addition, according to FAO et al. (2003), plants that are well supplied with potassium are less susceptible to disease. However, Huber & Thompson (2007) as well as Walters & Bingham (2007) have shown that an increase in fertilizer applications can,

depending on the mineral element the plant and the disease considered, increase or decrease the incidence and severity symptoms. Bruulsema et al. (2012) stated that only balanced nutrition with optimum levels of each nutrient could lead to the reduction of a disease. The severity values (AUSPC) recorded in the field in 2016 (446.25 to 586.87) are much higher than those recorded in 2015 are (72.7 to 135.83) on the same site. This large variation would be because the 2015 crop residues and the weeds present on the site would have constituted a host for the fungus during the installation of the new trial in 2016, which would have favoured a large and strong dissemination of disease. Indeed, the fungus (*H. turcicum*) is preserved during the dry season on crop residues in the form of spores (Ramathani, 2010), mycelium, conidia or chlamydo spores (Agrios, 1997). In addition, according to Esele (1995), hosts for *H. turcicum* include maize (*Zea mays* L.), sorghum (*Sorghum bicolor*), Sudan grass (*Sorghum sudanese*), Johnson grass (*Sorghum halepense*) and other herb species. Thus, at the beginning of the season, the fungus sporulates on crop residues and the spores are then disseminated by the wind over long distances, thus infecting cultivated plants. Once a lesion develops on a leaf, the fungus produces spores that can infect more tissue in the leaves (Gregory, 2004). There is no significant difference between the severity values (AUSPC) of Northern Corn Leaf Blight both at the level

of the varieties nor at the level of the combination of varieties \* K rates. This could be because varieties DMRESR-W, EVDT-97-STR and FAABA/QPM9 are susceptible to *H. turcicum* infection. Indeed, Wisser *et al.* (2011) and Chung *et al.* (2011) reported that complete resistance in maize to *Exserohilum turcicum* is rare whereas Zhang *et al.* (2020) found several lines that were completely resistant and identified 113 candidate genes with known roles in plant defense. Northern Corn Leaf Blight did not affect the grain yields of the different

varieties used for the trial. Indeed, the varieties have fully expressed their potential in terms of grain yield during the two years (2015 and 2016). As proof, the yield values obtained correspond to or are higher than the yields in a farming environment predicted by research for each of the varieties tested. According to Ahoyo Adjovi *et al.* (2013), yields in rural areas are respectively around 2.5 to 3t/ha for the varieties DMRESR-W and EVDT-97-STR but from 3 to 4t/ha for FAABA/QPM9.

## CONCLUSION AND APPLICATION OF RESULTS

The study reveals that the application of potassium decreases the severity of *Helminthosporium* leaf blight in maize. In addition, the rate K<sub>60</sub> is the one effective in control of Northern Corn Leaf Blight in the field. However,

there is a need for further studies in the greenhouse. Thus, the availability of fertilizer at the K<sub>60</sub> to farmers in the endemic zones could help for sustainable management of Northern Leaf Blight in maize.

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