

## Management of wheat rusts at different growth stages using Nativo 300 SC (trifloxystrobin 100g/L+tebuconazole 200g/L) fungicide

Ruth Wanyera<sup>1</sup>, Mercy Wamalwa<sup>2</sup>, Mercy Odemba<sup>2</sup>, Hannington Wanga<sup>1</sup>, Philister Kinyanjui<sup>1</sup>, Victoria Onyango<sup>2</sup>, James Owuoché<sup>2</sup>

<sup>1</sup>Kenya Agricultural and Livestock Research Organization, P.O. Private Bag, Njoro 20107, Kenya

<sup>2</sup>Egerton University, P. O Box 536, Egerton, Kenya

\*Corresponding author: wanyera@plantprotection.co.ke/wanyerar@gmail.com

### Abstract

Rusts (stem, yellow and leaf) remain the most important diseases of wheat worldwide due to their ability to cause severe yield losses in susceptible varieties. Timing of fungicide application in the integrated management of rusts on wheat is critical. This study was conducted to determine the wheat growth stage at which fungicide application can effectively control/reduce the damages caused by rust diseases. Field experiments were conducted in 2013 and 2014 in three locations; Njoro, Eldoret and MauNarok. Three doses of Nativo 300 SC (trifloxystrobin 100g/L+tebuconazole 200g/L) fungicide were applied at seven different growth stages of wheat variety Duma as split plot arrangement using a randomized complete block design with three replications. Results showed significant ( $P \leq 0.01$ ) differences among growth stages for stem rust infection, grain yield, grain and test weights. Area Under Disease Progress Curve (AUDPC) was significant for stem and yellow rusts in MauNarok and for leaf rust in Njoro. Significant ( $P \leq 0.01$ ) effects of environments and fungicide were observed on grain yield, grain and test weights. Environment  $\times$  growth stage interaction were significant ( $P \leq 0.01$ ) on AUDPC for stem rust, grain yield, grain and test weights. Fungicide application at tillering and flowering growth stages (GS) increased grain yield by 66.3%, grain weight by 41.6% and test weight by 17.27%. Therefore, wheat rusts can effectively be controlled by applying fungicide at tillering and flowering growth stages.

**Keywords:** Fungicide; Growth stage; Rust; Yield.

**Abbreviations:** AUDPC\_Area Under Disease Progress Curve; Ug99 (TTKSK)\_Stem rust race; GS\_Growth stage; RWA\_Russian Wheat Aphid; BYD\_Barley yellow dwarf.

### Introduction

Among the wheat (*Triticum aestivum* L.) diseases, stem (*Puccinia graminis*), leaf (*Puccinia triticina*) and yellow (*Puccinia striiformis*) rusts remain the most important in wheat production. This is due to their wide distribution, ability to form new races that can overcome the resistance in the newly released varieties, long distance migration and potential to develop rapidly under optimal environmental conditions resulting in severe yield losses (Roelfs, 1992; Kolmer et al., 2009). The initial epidemic of rusts on wheat was reported in Kenya as early as 1908 (Thorpe, 1959).

Stem (black) rust is a significant disease of wheat worldwide which can cause yield losses of up to 100% in susceptible varieties under conducive environment (Roelfs, 1985a; Leonard and Szabo, 2005). Wheat rust race evolution is a threat to global wheat production, for instance, a new stem rust race Ug99 (TTKSK), identified in Uganda in 1999 (Pretorius et al., 2000), is of great concern because it has broad virulence to most stem rust (*Sr*) genes (Jin et al., 2007). About one billion people reside in the anticipated path of Ug99 and most of them consume wheat produced within their borders (Olson, 2012). Although genetic resistance is the most effective method of controlling wheat rusts worldwide stem rust still remains a major threat to global wheat production and food security (Singh and Rajaram, 2002; Singh et al., 2011).

Wheat yellow (stripe) rust is also a significant disease of wheat worldwide (Chen, 2005; Hovmoller et al., 2010; Wellings, 2011). Yellow rust epidemic has been reported in South Africa, Kansas, China and Central Asia (Morgounov et al, 2004; Wan et al., 2004; Chen et al., 2005). The disease can cause significant reductions in both grain quality and yield in susceptible varieties (Dimmock and Gooding, 2002). Yield losses of up to 71% have been reported in Ethiopia (Worku, 2014). In Kenya yellow rust has remained an important disease since 1908 and no commercial variety is resistant (Bonthuis, 1985; KARI, 1990-2012). Yellow rust occurs in temperate regions with cool and moist-weather for spore germination, infection and survival (Danial et al., 1994; Chen, 2005). The pathogen is a global problem evolving into different races. In Kenya it is prevalent in the Rift valley region (Danial et al., 1994). Attacks by the pathogen occur annually and newly introduced resistant varieties lose their resistance within a short time.

Wheat leaf (brown) rust is the most common and widely distributed of the three wheat rusts and occurs in more regions more often than stem rust and stripe rust (Cherukuri et al., 2005; Kolmer, 2005; Kolmer, 2013). Yield losses caused by leaf rust have been reported to range from 5% to 16% on average, and up to 40% in epidemic years (Bolton et al., 2008). In Kenya, the disease appeared sporadically and has not been a problem for the past 20 years, but it has

recently emerged in the wheat fields and experimental plots (KARI, 2011). Understanding plant disease epidemiology and the role of practical approaches in disease evaluation is important in the development of management strategies (Jeger, 2004). Although wheat rusts are widely studied plant diseases, more knowledge is required to optimize their management in Kenya where they continue to be among the major factors limiting wheat production. The use of genetic resistance in controlling wheat rusts has been adopted worldwide. However, the problem of race evolution still persists and therefore the resistance of released varieties breaks down within a short period. This means that breeding programmes must continue to develop new resistant varieties with an increasing broad and stable type of resistance. This takes too long to accomplish. The ineffectiveness of resistance to new races necessitates new interventions in the management of the rusts. Therefore, the use of foliar fungicides as alternative management strategy for rust diseases should be adopted to maintain wheat production before the release of new resistant varieties. The importance of proper timing of chemical applications to control rust has been emphasized repeatedly (Hobbs, 1966; Walter *et al.*, 2007). Timing of fungicide application to reduce/control wheat rust is not properly understood in Kenya. Therefore this study was conducted to determine the growth stage of wheat at which fungicides can be applied to control/reduce rust infection.

## Results

In 2013, stem rust infection was observed across the three locations. Infection of yellow rust on the leaves was mainly observed in Njoro and MauNarok, while infection on the glumes was noted only in MauNarok. Leaf rust infection occurred mainly in Njoro in 2014. The effects of fungicide rates were not significant on the AUDPC for stem rust, while the effects of environment and growth stage were significant ( $P \leq 0.01$ ) for AUDPC, grain yield, a thousand grain weight and test weight. Significant ( $P \leq 0.01$ ) interaction effects (environment  $\times$  fungicide and environment  $\times$  growth stage) were observed for AUDPC, grain and test weight. In addition, the environment  $\times$  growth stage interaction effects were significant ( $P \leq 0.01$ ) for grain yield (Table 1).

### AUDPC for Stem rust and yellow rust

Among the environments, Eldoret had the highest AUDPC mean for stem rust while the means for Njoro 2013, Njoro and MauNarok 2014 were not significantly different (Table 3). Plots sprayed with Nativo 300 EC (trifloxystrobin 100 g/L+tebuconazole 200 g/L) at the rate of 1.25L ha<sup>-1</sup> showed a reduction in AUDPC compared to the wheat plots that were sprayed at the rate of 1.0L ha<sup>-1</sup> and 0.75L ha<sup>-1</sup>, respectively. Plots sprayed at tillering (20-29) GS and booting (40-49) GS; tillering (20-29) GS and flowering (60-69) GS had the lowest AUDPC for stem rust (81.79 and 65.99, respectively) (Table 3) while plots that were sprayed at flowering (60-69) GS had the highest AUDPC for stem rust (Table 3). Non-significant ( $P \leq 0.05$ ) effects of the fungicide treatment rates for yellow rust on the leaves and glumes were noted at Njoro and MauNarok in 2013 (Table 4). Infection of yellow rust on the glumes was significantly controlled when spraying was done at tillering (20-29) GS and booting (40-49) GS; tillering (20-29) GS and flowering (60-69) GS; first spray at 5% infection and 2 weeks later (Table 4). Infection of leaf rust was reduced in plots that were sprayed at the rate of 0.75L ha<sup>-1</sup> and the highest leaf rust infection was observed in plots that were sprayed at booting (40-49) GS. However, leaf rust

infection was low in plots sprayed at tillering (20-29) GS; tillering (20-29) and flowering (60-69) GS (Table 4).

### Grain yield

The environment and growth stage of the crop at which different fungicide rates were applied influenced grain yield. In 2013, mean grain yield for Njoro (3.36t ha<sup>-1</sup>) was the highest compared to the other three environments (Table 3). Application of the fungicide at the rate of 1.25L ha<sup>-1</sup> increased grain yield by 10.3% compared to the plots that were sprayed at the rate of 0.75L ha<sup>-1</sup>. Spraying at the rates of 0.75L ha<sup>-1</sup> and 1.0L ha<sup>-1</sup> did not show any comparative advantage on grain yield. Plots that were sprayed at tillering (20-29) GS and flowering (60-69) GS had grain yield increase of 66.3% compared to the control (unsprayed) plots. Fungicide application when the rust infection was at 5% and another spray two weeks later had a significant yield increase of 54.7%. In addition, fungicide application at tillering (20-29) GS and booting (40-49) GS had significant grain yield increase of 53.5% while spraying at flowering (60-69) GS had significant grain yield increase of 49.5% over the control (Table 3).

### Thousand grain weight

Among the test environments, the highest mean for thousand grain weight was observed at Njoro (38.39g) and Eldoret (38.77g) in 2013. Plots that were sprayed with the fungicide at the rate of 1.25L ha<sup>-1</sup> had grain weight advantage of 9.61% over plots that were sprayed with the fungicide at the rate of 0.75L ha<sup>-1</sup>. Plots that were sprayed at tillering (20-29) GS and flowering (60-69) GS and when the infection was at 5% followed by another spray two weeks later had a thousand grain weight of 39.6g and 38.69g which showed an increase of 41.6% and 38.67%, respectively, over the control plots (Table 3).

### Test weight

The mean test weight for the four environments was not significantly different. The lowest test weight (60.75 Kg HL) was observed at Njoro in 2014. Plots that received the fungicide at the rate of 1.25L ha<sup>-1</sup> had increased test weight of 4.61% compared to plots that received the fungicide at the rate 0.75 and 1.0L ha<sup>-1</sup>. Plots that were sprayed when the rust infection was at 5% followed by another spray two weeks later and tillering (20-29) GS and flowering (60-69) GS had the higher test weight of 73.13 and 72.92 with an increase of 17.56% and 17.27% over the control, respectively (Table 3).

### Regression

From the stepwise regression analysis, yellow rust infection on the glumes at milk (70-79) GS contributed to grain yield reduction ( $R^2=0.85$ ) in MauNarok (Table 5). In addition, yellow rust infection on the glumes at dough (80-90) GS contributed to the reduction of a thousand grain weight ( $R^2=0.92$ ) and test weight ( $R^2=0.91$ ). However, stem rust infection was detected as the major cause of grain yield ( $R^2=0.85$ ) and test weight ( $R^2=0.93$ ) reduction at flowering (60-69) GS in 2013 at Njoro. The infection of stem rust at milk (70-79) GS had also the greatest effect on reduction of thousand grain weight ( $R^2=0.94$ ) at the same site. In the subsequent season at Njoro and Eldoret, stem rust infection contributed to the reduction of grain yield ( $R^2=0.91$ ;

**Table 1.** Coordinates, elevation, mean temperature and annual rainfall for the experimental locations.

Location	Coordinates	Elevation (MASL)	Temperature (°C)	Rainfall (mm)
Njoro	0° 20' S, 35° 56'E	2185	9.7 (min), 23.5 (max)	900
Eldoret	0° 31' N, 35°15' E	2180	12 (min), 23 (max)	1,250
MauNarok	0°39' S, 35° 57' E	2754	6-14 (min), 22-26 (max)	1,200-1,400

Source: Jaetzold et al., 2005

**Table 2.** Mean squares from combined analysis for Area Under Disease Progress Curve (AUDPC) for stem rust, grain yield, thousand grain weight and test weight across four locations (Njoro 2013, Eldoret2013, MauNarok 2013, Njoro 2014).

Source	Df	AUDPC	Grain yield	Grain weight	Test weight
Environment	3	15579.73**	84302512.20**	1062.39**	54478.20**
Replicate within Environment	8	4912.02	175796.6	27.37	384.85
Fungicide	2	4912.01	344667.5*	218.64**	5328.49**
Environment × fungicide	6	7999.47**	152437.6	67.71**	2340.45**
Replicate × fungicide within Environment	16	5246.67	120425.8	12.89	434.46
Growth Stage	6	80151.32**	1511692.7**	538.16**	12610.08**
Environment × Growth Stage	18	16433.23**	428089.1**	49.5**	2212.14**
Fungicide × Growth Stage	12	3082.54	53216.4	13.99*	256.10
Environment × fungicide × stage	36	2019.09	73887.1	4.34	137.29
Cv		39.59	22.79	7.68	4.57

\*\* and \* significant at P&lt;0.01 and P&lt;0.05, respectively.

**Table 3.** Mean comparison for four locations, fungicide rates and growth stage for AUDPC for stem rust, grain yield, thousand grain weight and test weight on wheat variety Duma.

Environment	AUDPC	Grain yield (Ton ha <sup>-1</sup> )	Grain weight (g)	Test eight(HL Kg <sup>-1</sup> )
Eldoret	143.49a	0.76 b	38.39a	72.58a
Njoro 2013	123.92b	3.36 a	38.77a	72.79a
Mau Narok	114.42b	0.87b	32.89	72.09a
Njoro 2014	107.19b	0.77 b	30.47 c	60.75b
Lsd <sub>0.05</sub>	17.05	0.12	0.95	5.59
Mean	122.26	1.44	35.13	69.55
Fungicide		Grain yield gain (%)	Grain weight gain (%)	Test weight gain (%)
0.75L/ha	128.89a	1.37b	-	67.83c
1.0L/ha	124.0ab	1.43b	4.38	69.88b
1.25L/ha	113.89b	1.52a	10.93	70.96a
LSD <sub>0.05</sub>	14.76	0.10	0.82	4.85
Growth Stage (GS)				
Control	202.72a	1.01d	-	62.21e
First spray at 5% infection and 2 weeks later	111.18c	1.59ab	57.43	73.13a
Sprayed at tillering GS	100.24cd	1.27c	25.74	67.97d

**Table 3.** (Continued).

Growth Stage (GS)	AUDPC	Grain yield (Ton ha <sup>-1</sup> )	Grain yield gain (%)	Grain weight (g)	Grain Weight gain (%)	Test weight (HL Kg <sup>-1</sup> )	Test weight gain (%)
Sprayed at booting GS	135.71b	1.47b	45.54	34.45cd	23.48	69.11cd	11.09
Sprayed at flowering	158.19b	1.51b	49.50	36.35 b	30.29	71.06 b	14.22
Sprayed at tillering and booting GS	81.79de	1.55ab	53.47	35.70 bc	27.96	70.46bc	13.26
Sprayed at tillering and flowering GS	65.99e	1.68a	66.34	39.52a	41.65	72.92 a	17.27
LSD <sub>0.05</sub>	20.55	0.15	=	1.26	=	7.4	=

Grain yield gain (%); Grain Weight gain (%); Test weight gain (%) = (treated-untreated) x100/treated  
Means with different letters are significantly different at P< 0.05 within a column.

**Table 4.** Means of fungicide rates and growth stage for AUDPC for yellow rust (Yr) on the leaves ,glumes (1,2 ) and leaf rust (Lr) in Njoro and Mau Narok 2013.

Fungicide	Njoro		MauNarok		
	Yr	Lr	Yr	Yr on the glumes	
			AUDPC	1	2
0.75L/ha	1.0a	1.6b	175.83a	17.14a	25.71a
1.0L/ha	1.2a	3.95a	159.36a	17.86a	24.28ab
1.25L/ha	1.0a	2.86ab	164.50a	15.71a	21.43b
Lsd <sub>0.05</sub>	0.32	1.34	45.09	2.45	4.00
Growth Stage (GS)					
Control	1.0a	5.22ab	245.17a	27.78a	35.56a
First spray at 5% infection and 2 weeks later	1.0a	1.0c	238.06a	6.11d	9.44e
Sprayed at tillering (GS20-29)	1.0a	1.0c	102.11b	22.22b	32.78ab
Sprayed at booting (GS40-49)	1.0a	7.0a	211.00a	13.33c	20.56d
Sprayed at flowering (GS60-69)	1.0a	3.3b	231.83a	21.11b	27.78cb
Sprayed at tillering and booting	1.4a	1.0c	43.67b	12.78c	22.22cd
Sprayed at tillering and flowering	1.0a	1.0c	94.11b	15.00c	18.33d
Lsd <sub>0.05</sub>	0.48	2.04	68.88	3.75	6.12

Yr on the glumes1 (first severity notes1); Yr on the glumes 2 (second severity notes 2) at an interval of 1 week one. Means with different letters are significantly different at P< 0.05 within a column.

**Table 5.** Stepwise regression analysis: Effects of stem rust, yellow (stripe rust), and leaf rust on grain yield, thousand grain weight and test weight on wheat variety Duma at Mau Narok, Njoro and Eldoret.

Mau Narok	Variable	Parameter estimate	Standard error	Partial $R^2$	Model		
Grain Yield	Intercept	1129.05	69.4921				
	Milk GS	-20.3859	3.8218	0.8505	0.8505		
Grain weight	Intercept	37.77	0.049				
	Hard dough GS	-0.5284	0.0038	0.9229	0.9229		
	Milk GS	-1.0082	0.0074	0.0341	0.9570		
	Soft dough	1.0318	0.0083	0.0301	0.9871		
	Milk growth stage	0.5981	0.0089	0.0111	0.9982		
	Endof flowering GS	-0.0624	0.0023	0.0018	1.0		
Test weight	Intercept	382.83	6.63				
	Hard dough	-2.6033	0.3225	0.9136	0.9136		
	Milk GS	-6.8956	1.1730	0.0249	0.9385		
	Soft dough GS	1.8765	0.4136	0.0176	0.9560		
	Soft dough GS	5.4172	1.1690	0.0247	0.9807		
	Stem elongation GS	2.1354	0.7126	0.0174	0.9981		
Njoro 2013	Grain Yield	Intercept	3086.21	59.31			
		Endof flowering GS	-86.3600	15.3204	0.8572	0.8572	
		Heading GS	186.6127	13.0589	0.1285	0.9856	
		Milk GS	-51.8014	9.7331	0.0097	0.9953	
		Tillering GS	-27.0485	7.9075	0.0040	0.9993	
	Grain weight	Intercept	43.8614	0.068			
		Milk GS	-1.1722	0.0044	0.9465	0.9465	
		Grain filling GS	1.1619	0.0043	0.0420	0.9886	
		Heading GS	-0.2452	0.0018	0.0066	0.9952	
		End of flowering	-0.4597	0.0060	0.0038	0.9989	
		Tillering	0.6191	0.0137	0.0011	1.0	
	Test weight	Intercept	409.056	0.14			
		Tercept					
		Flowering GS	-4.6786	0.0063	0.9312	0.9312	
		Flowering GS	2.4169	0.0073	0.0409	0.9720	
Tillering GS		-4.9755	0.0348	0.0125	0.9845		
Heading GS		0.8060	0.0049	0.0155	1.0		
		0.6094	0.0763	0.0	1.0		
Njoro 2014	Grain Yield	Intercept	1182.94	36.67			
		Dough GS	-8.7596	0.7959	0.9118	0.9118	
		Heading GS	3.5514	15.8651	0.0629	0.9746	
		FloweringGS	23.7074	6.2820	0.0117	0.9864	
		BootGS	-50.6238	20.4478	0.0038	0.9901	
		Endofflowering(GS)	-15.4960	6.7743	0.0083	0.9984	
	Grain Weight	Intercept	35.47	3.89			
		Dough GS	-0.0919	0.0871	0.9655	0.9655	
		Milky GS	0.3623	0.3558	0.0056	0.9711	
		HeadingGS	2.2611	1.0403	0.0092	0.9803	
		Watery GS	-1.1962	0.7565	0.0083	0.9887	
	Njoro 2014	Variable	Parameter estimate	Standard error	Partial $R^2$	Model	
			Boot GS	-1.0439	0.8006	0.0071	0.9958
		Test Weight	Intercept	365.35	1.84		
Dough GS			-1.5741	0.1922	0.9395	0.9395	
Milky GS			-3.2711	0.7674	0.0300	0.9695	
Fowering GS			4.4364	0.5040	0.0163	0.9858	
End flowering GS			-2.4854	0.5844	0.0112	0.9970	
Milky GS			1.9248	0.7347	0.0026	0.9996	
Eldoret		Grain Yield	Intercept	728.1422	27.37		
			Flowering GS	-4.1574	2.1806	0.4210	0.4210
		Grain Weight	Intercept	41.63	0.69		
			Heading GS	-0.2185	0.1393	0.7904	0.7904
		Test Weight	Flowering GS	-0.1636	0.0714	0.0798	0.8703
			Intercept	369.26	2.094		
Test Weight		Heading GS	-0.5899	0.4210	0.6555	0.6555	
	Flowering GS	-0.2280	0.2158	0.0752	0.7306		

GS -Growth stage.

$R^2=0.42$ ), thousand grain weight ( $R^2=0.96$ ;  $R^2=0.79$ ) and test weight ( $R^2=0.93$ ;  $R^2=0.65$ ).

## Discussion

Foliar fungicides are used to manage diseases caused by fungi in wheat in order to achieve high yield and quality. Fungicides do not give a 'yield bump' rather they protect yield potential that is already built into the crop. This protection can only be achieved if attention is paid to the details of application of fungicide. Not all the three rusts were observed in the three locations where the experiments were conducted. This could probably be due to unfavourable environmental conditions for disease infection and development.

Effects due to environments and fungicide on AUDPC for stem rust and grain yield suggest that there was variation among the test environments and the rates at which the fungicides were applied. Significant environment  $\times$  fungicide indicates that the effect of fungicide on the rate of application also varied with environment. Plots that had the highest AUDPC had low grain yield, thousand grain and test weights. In a different study on barley, Ochoa and Parlevliet (2007) found out that yield loss due to leaf rust was related to AUDPC. Timing of fungicide application is critical for control of fungal diseases (Walter et al., 2007). In this study, spraying at tillering and flowering growth stages resulted in the reduction of stem rust infection. Similar studies on timing for foliar fungicide application concluded that the best time for application was at early anthesis (Wiersma and Motteberg, 2005; D'Angelo et al., 2014).

Results showed that there were environmental effects on AUDPC for stem rust, grain yield, grain weight and test weight. The variations in environment on AUDPC for stem rust were probably due to weather related factors that prevailed at the experimental sites. In this study, three locations Njoro, Eldoret and MauNarok were selected because of favourable weather conditions for rust infection and development. Generally Njoro and Eldoret have hot days of 23 °C -30°C, mild night temperatures below 15°C and adequate moisture for night dews. MauNarok often experiences cool weather, frequent rainfall during the growth seasons which is highly favourable for yellow rust infection and development (Wanyera et al., 2009). The warm moist conditions in Eldoret favoured stem rust infection hence the high AUDPC for stem rust. A study on the effect of Amistar 250 EC (azoxystrobin 250 g/L) and Dithane M-45 (mancozeb 800g/Kg) on growth parameters of maize showed that an increase in the concentration of the fungicide led to increase in growth parameters of maize seedling (Aisha, 2014). Increasing the rate of Nativo fungicide to 1.25L ha<sup>-1</sup> reduced the rust severity, hence the reduction in AUDPC.

It was evident that grain yield increased with the increase in the rate of fungicide application. This increment is attributed to the reduction of stem rust destruction which results to premature death of spikelets or entire spikes hence the development of shrivelled grains. The yield increase due to fungicide applications could also be partially due to phytonic effect of fungicides. This stimulatory effect of fungicide treatments on growth may result in significant yield increases even in the absence of the disease (Wegulo et al., 1998). In the United Kingdom, application of fungicides to winter wheat resulted in a yield response of up to 89% (Cook et al., 1999). Rust infection at tillering GS had greater effects on grain yield, a thousand grain and test weights reduction across the three environments. Kolmer et al. (2005) findings

showed that greater yield losses result from infections before the jointing and tillering growth stages.

Wheat rusts interfere with photosynthetic activities and reduce the mobilization of the carbohydrates to the kernels. Increasing the fungicide rate restricted the development of rust uredinia hence reducing the destruction of the photosynthetic area. The low grain yield, grain and test weights at the rate of 0.75L ha<sup>-1</sup> was attributed to the reduction of the photosynthetic area and destruction of the phloem tissues on variety Duma. Effects of fungicide rates and growth stages on plots infected with yellow rust on the glumes were noted at Njoro in 2013 and MauNarok in 2014. Increase of application rates from 0.75 L ha<sup>-1</sup> to 1.25L ha<sup>-1</sup> reduced yellow rust infection on the glumes by 17%. This was also observed on stem rust when application rates were. Infection of yellow rust on the glumes at milk stage contributed to the reduction of grain yield. In contrast to yellow rust, stem rust infection had greatest effect in the reduction of grain and test weight at dough stage, leading reducing grain yield.

The effects of stem rust on grain yield varied across environments. In 2013, stem rust infection contributed to the reduction of grain yield and thousand grain weight at Njoro. Lackermann et al. (2011) found that both location and disease contributed to the reduction of grain yield. Grain yield, thousand grain weight and test weight were highly affected by the rust infection at different growth stages. In Eldoret, stem rust infection at the dough growth stage highly contributed towards yield reduction. Therefore, timely detection of rusts and application of fungicides are important factors in controlling wheat rusts (Roelfs, 1985b; Beard et al., 2004).

## Materials and Methods

### Experimental sites

Field experiments were conducted in 2013 and 2014 in three locations (Table 1).

### Plant material and Fungicide

Wheat commercial variety Duma from KALRO-Njoro which is highly to moderately susceptible to stem, leaf and yellow rust was used. A commercial foliar fungicide Nativo 300 SC (trifloxystrobin 100g/L+tebuconazole 200g/L) was used. Nativo 300 SC is a systemic broad spectrum fungicide which controls fungal diseases and improves quality and yield of crops. The fungicide was obtained from Bayer East Africa Limited.

### Experimental procedures

Wheat variety Duma was planted for evaluation in a randomized complete block design (RCBD), split-plot arrangement with three replications in the three locations. Plot size was 9 m<sup>2</sup> with a length of 6 m and an inter-row spacing of 0.2 m. Sowing was done by an experimental drill at a seeding rate of 125Kg ha<sup>-1</sup> in May 20 and 21, 2013 at Njoro; October 3 and 4, 2013 at MauNarok; May 14, 2014 at Eldoret and June 23, 2014 at Njoro. Di-ammonium phosphate (D.A.P, 18% N, 46% P, 0% K) fertilizer was used at the recommended rate of 150 Kg ha<sup>-1</sup> at planting. Hussar Evolution (fenoxaprop-p-ethyl 64g/L +idosulfuron methyl sodium 8g/L +mefenpyr-diethyl 24g/L) was applied as post-emergence herbicide at 1.0L ha<sup>-1</sup> for the control of both grass and broadleaved weeds. Another application of Buctril MC

(bromoxynil+ MCPA) labelled for control of broad-leaved weeds was applied at the rate of 1.25L ha<sup>-1</sup> at tillering (20-29) GS. Thunder OD 145 (imidachloprid 100g/L +beta-cyfluthrin 45g/L) insecticide was applied at the rate of 0.3L ha<sup>-1</sup> at tillering (20-29) GS, flowering (60-69) GS and milk (70-79) GS to control Russian Wheat Aphid (RWA) and other aphid vectors for barley yellow dwarf disease (BYD). The growth stages were based on Zadocks et al. (1974) scale. Nativo 300 SC (trifloxystrobin 100 g/L+tebuconazole 200 g/L) was applied at the rates of 0.75L ha<sup>-1</sup>, 1.0 L ha<sup>-1</sup> and 1.25 L ha<sup>-1</sup>. The untreated plots served as the control. The fungicide was applied using a knap sack sprayer at a recommended water volume of 200 Lha<sup>-1</sup> at six different stages (1. First spray at 5% infection and 2 weeks later, 2. Tillering, 3. Booting, 4. Flowering, 5. Tillering and booting, 6. Tillering and flowering).

### Data collection

Rust severities in the plots were based on the modified Cobb scale (Peterson et al., 1948), on a weekly basis before and after fungicide application. At maturity, each plot was harvested by hand using sickles and threshed by a stationery thresher (Model LPT D, serial no. T09235 ALMACO USA. Specialized agricultural equipment. NEVADA Iowa, USA). Harvesting dates for Njoro 2013, 2014; MauNarok 2013/2014 and Eldoret were October 17-18, 2013, November 17-19, 2014; April 1, 2014; and October 17, 2014, respectively. Grain weight measurements were taken after cleaning and drying to moisture content of 13%-14%. A sample of grain was taken from each plot for determination of grain weight based on the weight of a thousand grains and test weight (a measure of the density).

### Statistical analysis

Area under Disease Progress Curve (AUDPC) was calculated by a computer programme developed at the International Centre for Maize and Wheat Improvement (CIMMYT). AUDPC for combined analysis was calculated for stem rust in all environments. For yellow and leaf rusts, the ANOVA for each environment was used and means separated by LSD at (P≤0.05). The analysis was done using the following statistical formula:

$$Y_{ijk} = \mu + E_i + R_{j(i)} + F_k + EF_{ik} + FR_{jk(i)} + S_l + ES_{(il)} + FS_{(kl)} + FSE_{(ikl)} + \varepsilon_{ijklm}$$

: Where  $\mu$  = mean of the observations,  $E_i$  = the effect of the  $i^{th}$  environment,  $R_{j(i)}$  = effect of the  $j^{th}$  replicate within  $i^{th}$  environment,  $EF_{ik}$  = effect due to the interaction between  $i^{th}$  environment and the  $k^{th}$  fungicide rate within  $i^{th}$  environment and  $j^{th}$  replicate,  $F_k$  = effect of the  $k^{th}$  fungicide rate within  $i^{th}$  environment and  $j^{th}$  replicate,  $S_l$  = the effect of the  $l^{th}$  stage of application of fungicide in the  $j^{th}$  replicate in the within  $i^{th}$  environment.  $ES_{il}$  = effect due to the interaction between  $i^{th}$  environment and  $l^{th}$  stage of application of fungicide in the  $j^{th}$  replicate,  $FS_{kl}$  = effect due to the interaction between  $k^{th}$  fungicide rate and  $l^{th}$  stage of application of fungicide in the  $j^{th}$  replicate in the within  $i^{th}$  environment,  $FSE_{ikl}$  = effect due to the interaction of  $k^{th}$  fungicide rate within  $j^{th}$  replicate and  $l^{th}$  stage of application of fungicide in the  $j^{th}$  replicate and  $i^{th}$  environment;  $\varepsilon_{ijklm}$  = random error of the experiment.

Stepwise regression backward selection was used to determine the effects of rusts and growth stage on grain yield, a thousand grain weight, test weight using SAS (SAS 1999).

### Conclusion

Fungicides can significantly increase wheat grain yield by effectively reducing rust infection and development on susceptible varieties. The study showed that, growth stage is an important factor in the management of wheat rusts using fungicides. Tillering and flowering were the most appropriate growth stages at which Nativo 300 SC (trifloxystrobin 100g/L+tebuconazole 200g/L) foliar fungicide applied at the rate of 1.25 L ha<sup>-1</sup> effectively reduced/controlled the rust infection and increased grain yield. It was also noted that, early spraying when the disease was at 5% infection and 2 weeks later significantly reduced yellow and leaf rust. This study showed that foliar fungicides can be used to manage wheat rust as a short term control strategy and that Integrated Rust Disease Management will be an appropriate strategy for reducing the disease damage and maintaining grain yield of wheat in Kenya.

### Acknowledgements

The authors wish to acknowledge the World Bank for providing the study funds through the Eastern Agricultural Productivity Project (EAAPP).

### References

- Aisha Mohammed Homod Alrajhi (2014) Effects of amistar and dithane M-45 a systemic fungicide on growth parameters and antioxidative enzymes of maize (*Zea mays* L). Res Rev J Bot Sci ISSN: 2320-0189.
- Bolton MD, Kolmer JA, Garvin DF (2008) Wheat leaf rust caused by *Puccinia triticina*. Mol Plant Pathol 9: 563-575.
- Beard C, Jayasena K, Thomas G, Loughman R (2004). Managing stem rust of wheat. Farmnote 73, State of Western Australia.
- Bonthuis H (1985) Survival of stripe rust (*Puccinia striiformis*) on wheat in the Kenyan highlands and the consequences for virulence. Mededelingen Faculteit en bouwwetenschappen Rijks Universiteit. Gent 50:1109-1117.
- Chen XM (2005) Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. Plant Pathol 27:314-337.
- Cherukuri DP, Gupta SK, Charpe A, Koul S, Prabhu KV, Singh RB, Haq QMR (2005) Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. Euphytica 143: 19-26.
- Cook RJ, Hims MJ, Vaughan TB (1999) Effects of fungicide spray timing on winter wheat disease control. Plant Pathol 48:33-50.
- Daniel DL, Stubbs RW, Parlevliet JE (1994) Evolution of virulence patterns in yellow rust races and its implication for breeding for resistance in wheat in Kenya. Euphytica 80: 165-170.
- D'Angelo D L, Bradley C A, Ame, K A, Willyerd K T, Madden L V, Paul P A (2014) Efficacy of fungicide applications during and after anthesis against Fusarium head blight and deoxynivalenol in soft red winter wheat. Plant Dis. 98:1387-1397.
- Dimmock JPRE and Gooding MJ (2002) The influence of foliar diseases and their control by fungicides on the protein concentration in wheat grain: a review. J Agric Sci 138:4:349-366.
- Hobbs CD, Futrell MC (1966) Evaluation of nickel-plus dithiocarbamates for control of wheat stem rust. Plant Dis 50: 373-76.

- Hovmoller MS, Walter S, Justesen AF (2010) Escalating threat of wheat rusts. *Sciences* 329:369.
- Jaetzold R, Schmidt H (2005) Farm management handbook of Kenya 2nd edn. (Rift Valley). Ministry of Agriculture, Livestock and Marketing.
- Jeger MJ (2004) Analysis of disease progress as a basis for evaluating disease management practices. *Annu Rev Phytopathol* 42:61-82.
- Jin Y, Singh RP, Ward RW, Wanyera R, Kinyua M, Njau P, Fetch T, Pretorius ZA, Yahyaoui A (2007) Characterization of seedling infection types and plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f.sp. *tritici*. *Plant Dis* 91: 1096-1099.
- Kenya Agriculture Research Institute (2011) Annual reports. Nairobi, Kenya.
- Kenya Agriculture Research Institute (1990-2012) Annual reports. Nairobi, Kenya.
- Kolmer JA (2005) Tracking wheat rust on a continental scale. *Plant Biol* 8:441-449.
- Kolmer JA, Long DL, Hughes ME (2009) Physiologic specialization of *Puccinia triticina* on wheat in the United States. *Plant Dis*. 93:538-44.
- Kolmer JA (2013) Leaf rust of wheat: pathogen biology variation and host resistance. *Forests* 4: 70-84.
- Lackermann KV, Conley SP, Gaska JM, Martinka MJ, Esker PD (2011) Effect of location cultivar and diseases on grain yield of soft red winter wheat in Wisconsin. *Plant Dis* 95:1401-1406.
- Leonard KJ and Szabo LJ (2005) Stem rust of small grains and grasses caused by *Puccinia graminis*. *Mol Plant Pathol* 6:99-111.
- Leonard KJ, Szabo LJ (2015) Stem rust of small grains and grasses caused by *Puccinia graminis*. *Mol Plant Pathol* 6:99-111.
- Morgounov A, Yessimbekova M, Rsaliev S, Baboev S, Mumindjanov H and Djunusova M (2004) High-yielding winter wheat varieties resistant to yellow and leaf rust in Central and Asia. Proceedings of the 11<sup>th</sup> international cereal rusts and powdery mildew conference, 22-27 August 2004, John Innes Centre, Norwich, UK, European and Mediterranean cereal rust for Wageningen, Netherlands, cereal rusts and powdery mildew bulletin, A2. 52.
- Ochoa J, Parlevliet JE (2007) Effect of partial resistance to barley leaf rust, *Puccinia hordei* on the yield of three barley cultivars. *Euphytica* 153:309-312.
- Olson EL (2012) Broadening the wheat gene pool for stem rust resistance through genomic assisted introgressions from *Aegilops tauschii* PhD thesis, Kansas State University, Manhattan, Kansas.
- Peterson RF, Campbell AB, Hannah AE (1948) A diagrammatic scale for estimating rust intensity of leaves and stems of cereals. *Can J Res* 26:496-500.
- Pretorius ZA, Singh RP, Wagoire WW, Payne TS (2000) Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis* f.sp. *tritici* in Uganda. *Plant Dis* 84:203.
- Roelfs AP, Singh RP, Saari EE (1992) Rust diseases of wheat concepts and methods of disease management. Mexico DF CIMMYT.
- Roelfs AP (1985a) The cereal rusts Vol II diseases, distribution, epidemiology and control.
- Roelfs AP (1985b) The cereal rusts Vol II diseases distribution epidemiology and control.
- SAS Institute Inc (1999) SAS for windows, Version 9.00. Cary: NC, USA.
- Singh RP, Rajaram S (2002) Breeding for disease resistance in wheat FAO plant production and protection series, PP. 567.
- Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau, P, Herrera-Foessel S., Singh PK, Singh S, and Govindan V (2011) The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annu Rev Phytopathol*. 49:465-481.
- Thorpe HC (1959) Wheat breeding in Kenya. First international wheat genetics symposium, University of Manitoba, Canada.
- Walter M, Obanor FO, Smith JT, Ford C, Boyd -Wilson KSH, Harris-Virgin P, Langford GI (2007) Timing of fungicide application for *botrytis cinerea* control in blackcurrant (*Ribes nigrum*). *N Z Plant Prot* 60:114-122.
- Wan A, Zhao Zhong Hua X M, Chen, He Zhong Hu, Jin SheLin, Jia Qiu Zhen, Yao Ge, Yang Jia Xiu, Wang Bao Tong, Li GaoBao, Bi YunQing, Yuan Zong Ying ( 2004). Wheat stripe rust epidemic and virulence of *Puccinia striiformis* f. sp. *tritici* in China in 2002. *Plant Dis* 88: 896-904.
- Wanyera R, Macharia JK, Kilonzo SM, Kamundia JW (2009) Foliar fungicides to control wheat stem rust race TTKS (Ug99) in Kenya. *Plant Dis* 93: 929-932.
- Wellings C (2011). Global status of stripe rust: a review of historical and current threats. *Euphytica*, 179: 129-141.
- Wegulo SN, Yang XB and Martinson CA (1998). Soybean cultivar responses to *Sclerotinia sclerotiorum* in field and controlled environment studies. *Plant Dis* 82: 1264-1270.
- Wiersma JJ, Motteberg CD (2005) Evaluation of five fungicide application timings for control of leaf-spot diseases and Fusarium head blight in hard red spring wheat. *Can. J. Plant Pathol* 27:25-37.
- Worku D (2014) Epidemics of *Puccinia striiformis* f.sp.*tritici* in Arsi and West Arsi zones of Ethiopia in 2010 and identification of effective resistance genes. *Nat Sci Res* 4:2224-3186.
- Zadoks JC, Chang TT, Konazak CF (1974) A decimal code for growth stages of cereals. *Weed Res*14:415-421.