DETERMINATION OF WHEAT STEM RUST *(Puccinia graminis* Pers. f. sp. *tritici* Eriks & E. Henn*)* SEVERITY AND PHYSIOLOGICAL RACES IN UASIN GISHU KENYA AND THEIR CONTROL USING TEBUCONAZOLE + TRIDIMENOL

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A thesis submitted to the Graduate School in partial fulfillment of the requirements for the Degree of Master of Science in Agronomy (Crop Protection Option) of Egerton University

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ABSTRACT

New races of wheat stem rust, which are virulent to the previously resistant genes have made up to 90% of the worlds wheat to be at risk. East Africa region is the hot spot for evolution of these races. A study was carried out to determine the wheat stem rust severity, races and evaluate their control using tebuconazole 250g/L + tridimenol 125g/L fungicide. A field survey was carried out in Uasin Gishu region Kenya. Wheat stem rust severity was determined based on modified Cobs scale and races of *Puccinia graminis* f.sp. *tritici* were identified using the 20 North American wheat differentials. The races identified were screened on some previously resistant wheat stem rust differentials under greenhouse conditions. Virulence and avirulence was detected based on the reaction of the races on the leaves of the test differential. Wheat stem rust control was studied by evaluating three rates of tebuconazole 250g/L + tridimenol 125g/L at three different timing of application under a single spray regime. The data was analyzed using MSTATC, GENSTAT and SPSS statistical packages. Variation in stem rust severity was noted in the different zones, and a correlation analysis indicated that there was no significant correlation ($r=0.398$) of stem rust severity and race diversity. However, there was a significant ($P\leq0.05$) negative correlation ($r=-0.461$) of race diversity and altitude and a highly significant ($P\leq0.01$) negative correlation ($r=-0.713$) of altitude and stem rust severity. Four races were identified where the predominant race was TTKST, *(Ug99+Sr24)*. A new race PTKST *(Ug99+Sr24-Sr21)* was reported and was also widespread. The results revealed that race TTKST was virulent to five of the nine previously resistant differentials, and only *Sr 22, Sr 27, Sr 32* and *Sr 35* appeared to show resistance to this race. Race PTKST was virulent to two of the nine test differentials. Both race TTKSK and TTTSK were not virulent to any of the nine test differentials. The effect of tebuconazole 250g/L + tridimenol 125g/L rates and time of application revealed that a grain yield of more than 3 tones/ha can be achieved when a single spray of Silvacur is applied at either a rate of 1 L/ha when the initial disease severity is at 10% during the long rain season (high disease pressure) or a low rate of 0.75 L/ha applied at 10% during the short rain season (low disease pressure). The study, therefore established that; wheat stem rust is severe in this region, with new virulence and change in race dynamics noted, more so farmers should be sensitized on the need for proper rate and timing so as to reduce the spray frequencies thus reducing cost of production.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
CIMMYT	International Centre for Maize and Wheat Improvement
DAP	Days after planting
FAO	Food and Agriculture Organization
FDS	Final Disease Severity
GENSTAT	Generation statistics
IT	Infection Type
KARI	Kenya Agricultural Research Institute
LSD	Least Significant Difference
MSTATC	Masters of Statistics
MT	Metric tones
Pgt	*Puccinia graminis tritici*
R	Pearson coefficient of correlation (coefficient of correlation)
Sr	Stem rust resistance gene
SPSS	Statistical Package for Social Scientist
TKW	Thousand kernel weight

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Wheat is among the grain cereals which contribute significantly to food security in Kenya and ranks second after maize. The current area under wheat production is estimated at 150,000 ha. The consumption stands at 750,000 metric tons annually and this is expected to increase to 1,000,000 metric tons by 2015 (FAO, 2010). The demand for consumption rises at an estimated 7% per year, driven by population growth, increased urbanization and changing diets. The annual production is about 350,000MT, yet the demand stands at 750,000MT. This means the local production meets only 40% of the total consumption; hence Kenya imports 60% of its wheat requirements (FAO, 2010). While the yields have been improved significantly from 0.3% per year (1951-66) to over 1.7% per year in 1988-2000, it is now being threatened by the advent of new races of stem rust. Stem rust caused by *Puccinia graminis* f.sp. *tritici* is at present one of the major threats to wheat production, leading to heavy yield losses in Kenya (Njau *et al.,* 2009).

The environment in East African highlands enables large populations of stem rust to persist year round and this situation contributes to evolution of new physiological races. East Africa is known to be hot-spot for origin of new virulent races of rust (Singh *et al.,* 2006). In 2006, a variant of race Ug99 with added virulence on stem rust gene *Sr 24* further increased the vulnerability of wheat to stem rust worldwide (Jin *et al.,* 2008). Jin *et al* (2009) has also reported another variant of Ug99 (TTTKS) which is virulent to *Sr 36.* Severe disease severity has been observed at high altitudes, although traditionally the fungus normally occurs in low to mid-altitude areas (Wanyera, 2008a). Continual and current monitoring of the pathogen population is necessary so that resistance is effective at variety release (Singh *et al.,* 2006). The early recognition of new virulence leads to breeding of resistant varieties before the pathogen causes an epidemic (Park, 2008). It is anticipated that mutation toward more complex virulence will likely occur as the fungal population size increases and selection pressure is placed on the population by resistant varieties, thus the need to have an integrated approach to reduce the

inoculum pressure. Fungicides as foliar or seed treatments will play a role in the integrated management of wheat stem rust until new varieties with improved resistance are released. Effective control of stem rust can be achieved with standard application of fungicides (Wanyera *et al.,* 2009). There is, therefore, urgent need to develop economical spray regimes that farmers can adopt on some of the stem rust recommended fungicides. This study was, therefore, carried out to identify the physiological races of wheat stem rust in Uasin Gishu region, screen their virulence status on stem rust differentials and determine their control using tebuconazole 250g/L + tridimenol 125g/L.

1.2 Problem statement

Occurrence of race Ug99 which is virulent to *Sr 31* and the new mutants which are virulent to *Sr 24* and *Sr 36* in Kenya has made all local commercial wheat varieties susceptible. Studies on wheat stem rust race distribution in Kenya are limited. With the current mutants of race Ug99 being reported, breeders are not sure how this races are distributed in Kenya. There is continued fear that race Ug99 is still mutating as previously resistant materials are increasingly losing their resistance, thus the need for continued race surveys and screening the previously resistant materials against all the races reported.

Control of wheat stem rust is paramount, and as the farmers await varieties with good level of resistance, fungicides will continue to play an important role. Earlier manufactures' recommended spray programmes are no longer effective in control of new races of wheat stem rust. Although tebuconazole 250g/L+ tridimenol 125g/L has been found to effectively control race Ug99 (Wanyera *et al.,* 2009), optimal rates and timing and its interaction has not been studied, more so with the ever changing race dynamics. Chemicals are expensive and hazardous to the environment, thus the need for research to concentrate on few application frequencies, low rates and optimal timing for effective disease control.

1.3 Justification

The use of effective resistance has for long been the most economical way for control of wheat stem rust. In order to effectively utilize stem rust resistance in the improvement of wheat, it is

necessary to monitor the virulence composition and dynamics in the stem rust pathogen population. The dynamic nature of stem rust pathogen provides a continuous threat to released wheat varieties where some are rescinded soon after. Some new races are known to cause severe losses within a short time as it happened with race TTKST which was detected in 2006 and caused major losses in 2007 in Kenya. Others take some time to build enough inoculum to cause an epidemic like race Ug99 which is thought to have been present in Kenya since 1993, but it was not until 2004-2005 that farmers experienced epidemics. Although race Ug99 is known to be present in East Africa (Singh, 2006), the extent of its distribution and its frequency of occurrence is not well understood, more so with the recent detection of race Ug99 mutants. Timely identification of a race can assist the breeders to develop a resistant variety in time before an epidemic. The information on pathogenicity will enable pathologists and breeders to utilize the most effective resistance genes in their breeding programmes. The continued mutation of wheat stem rust makes screening of the previously resistant materials a continuous process, therefore any race identified should be screened on the resistant materials to determine its virulence.

As part of the integrated disease management, chemical control continues to play an important role in control of stem rust (GRI, 2006). Since the release of a variety takes many years, there is need to have effective fungicides, effective rates and affective timing of application. The use of fungicides has been reported to effectively control stem rust in wheat. In comparison with other wheat fungicides, tebuconazole 250g/L + tridimenol 125g/L is currently recommended for control of wheat stem rust. However, the high cost of chemicals application and environmental concerns require research to concentrate on minimum number of application frequencies, low rate and effective timing. Therefore, the need to screen promising fungicides under these conditions.

1.4 Objectives

1.4.1 Broad objective

To contribute to increased wheat production in Kenya by reducing its losses caused by wheat stem rust disease.

1.4.2 Specific objectives

i. To determine wheat stem rust severity and physiological races in Uasin Gishu region.

ii. To determine the virulence of stem rust races identified in Uasin Gishu region on current resistant wheat differentials.

iii. To determine the effect of rates and application time of tebuconazole 250g/L + tridimenol 125g/L in control of wheat stem rust.

1.5 Hypothesis (null)

i. Stem rust severity and races in Uasin Gishu region are not uniformly distributed.

ii. Wheat stem rust races in Uasin Gishu region are not virulent to current resistant wheat stem rust differentials.

iii. There is no effect of rates and application time of tebuconazole 250g/L + tridimenol 125g/L in control of wheat stem.

CHAPTER TWO

LITERATURE REVIEW

2.1 The stem rust pathogen

Stem rust, alongside leaf and yellow rusts, belong to the genus *Puccinia*. Specifically, stem rust which is also called black rust is caused by *Puccinia graminis* Pers. The name black rust is associated with the black teliospores that are produced towards the end of the growing season of an infected wheat plant (Roelfs, 1984; Agrios, 1997). *Puccinia graminis* is characterized by marked specialization on the host genera to produce formae specialis. Thus; *P. graminis* f. sp. *avenae*: specific to oats and some related grasses, *P. graminis* f. sp. *secalis*: specific to rye and some related grasses, *P. graminis* f. sp. *tritici*: specific to wheat, barley, and many of the relatives of wheat (Luig and Watson, 1976). A formae specialis does not produce pustules on a species outside its host range (Knott, 1989). The genus *Puccinia* is a member of the family pucciniaceae of the order uredinales of the class Basidiomycetes (Littlefield, 1981). In the basidiomycetes, meiosis occurs in a basidium and results in the production of four, haploid, single-celled basidiospores.

2.1.1 Life cycle of *Puccinia graminis* f.sp *tritici*

In most parts of the world the life cycle of *Puccinia graminis* f.sp *tritici* consists of continual uredinial generation, urediniospores are air borne and can move from one wheat plant to the other or from field to field (Watson and Sousa, 1983). Stem rust has five spore types and both sexual and asexual stages (Figure 1). The asexual stage occurs on wheat and related grasses and the sexual stages on an alternative host; barberry (*Berberis* spp) or *Mahonia* spp (Bowder, 1971). This rust is referred as being macrocyclic, since it has the five spore stages as well as heteroecious, since it has an alternate host. The alternate hosts are of significance mainly in areas with cold winters where the asexual stage cannot survive but the teliospores do (Leonard and Szabo, 2005). The pustules of stem rust that are seen on wheat during most of its growing cycle are called uredia and produce urediospores. Urediospores contain two genetically different nuclei, that are dikaryotic, oblong in shape and reddish brown in color. A tremendous number of

spores are produced by each individual uredium for several weeks. In the absence of strong wind current, most spores remain within the crop canopy and cause re-infection. On the contrary, wind carries spores sometimes to long distances. Luig (1985) observed that spores have been carried from southern Africa to Australia in at least three occasions. The more common phenomena involve movement of spores from field to field over short distances.

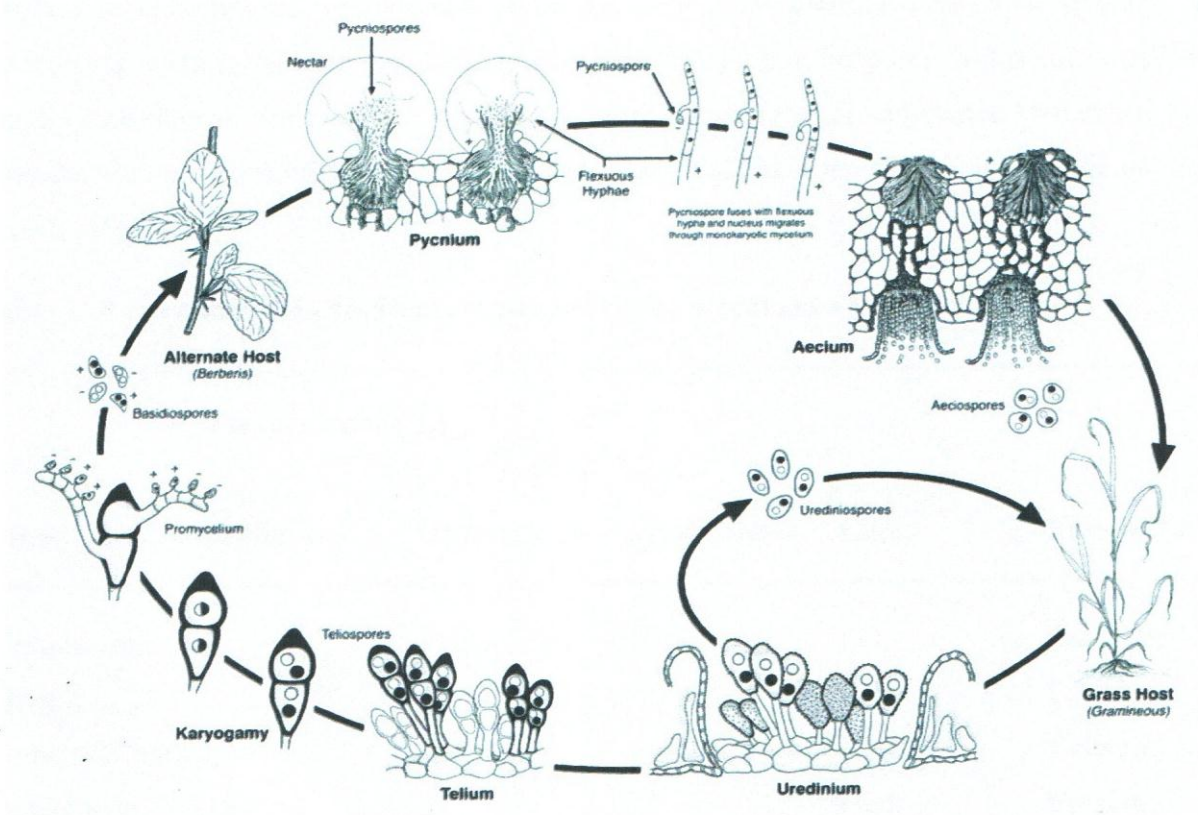


Figure 1: Reproduction cycle of *Puccinia graminis* (After Kurt and Szabo, 2005)

Urediospores germinate when free water is present on the wheat plants. Germ tubes grow until they reach a stoma. This is followed by the development of an appressorium over the stomatal opening, through which an infection peg pushes and a vesicle develops in the substomatal cavity (Staples and Macko, 1984). Infection hyphae grow from the vesicle and produce haustoria mother cells. Penetration pegs then push into the host cells and give rise to haustoria (Harder and Chong, 1984).

Infections usually become visible in 5-8 days and sporulation begins 7-14 days depending largely on temperature; 30°C is the optimum temperature for these events (Table 1). Where the alternate host is not known to exist, wheat stem rust can survive entirely in the asexual stage (Kurt and Szabo, 2005). As the growing season ends, the uredia begin to turn black as urediospore production stops and production of black teliospores follows. The teliospores are resistant to extremes of weather and germinate only after alternate periods of freezing and thawing, or wetting and drying. Teliospores give rise to basidiospores which are small and slightly oval shaped. Basidiospores are forcibly discharged in the air and carried to alternate host. They then germinate rapidly, produce an infection peg and penetrate directly into epidermal cells (Roelfs, 1985).

Table 1: Environmental conditions required for the wheat stem rust

	Temperature (°C)				
Stage	**Minimum**	**Optimum**	**Maximum**	**Light**	**Free water**
Germination	2	15-24	30	Low	Essential
Germling	-	20	-	Low	Essential
Appressorium	-	16-27	-	None	Essential
Penetration	15	29	35	High	Essential
Growth	5	30	40	High	None
Sporulation	15	30	40	High	None

(Roelf *et al.*, 1992)

Barberry genotypes may be either resistant or susceptible to particular stem rust races. Once infected, there is development of sub epidermal, flask-shaped pycnia on the upper leaf surface of barberry. In 7-14 days after infection the pycnia open and a viscous liquid (honey dew) containing pycniospores appears. The pycniospores function as male gametes. The pycnia are of two mating types, + and -. Successful mating can only occur between opposite types. Pycniospores are transferred from one pycnium to another by insects attracted to the honey dew

or by splashing raindrops. Ultimately, aeciospores are produced from the pycniospores which are then carried by wind to nearby wheat fields where they mate and initiate heavy, early infections. The infections on wheat develop into uredia, thus completing the life cycle (Roelfs, 1985; Leonard and Szabo, 2005).

2.1.2 Epidemiology of stem rust of wheat pathogen

The epidemic development of diseases within the growth cycle of the host is determined by the initial amount of disease, X_o, and the rate at which the disease increases, described as the apparent infection rate r (Van der plank, 1968). To reduce the severity of disease, the reproductive rate of the pathogen must be decreased. The major components of resistance which affect the reproduction of the pathogen, are a reduction in the infection frequency (IF) or lesion number, a lengthening of the latent period (LP), and decrease in spore production (SP).

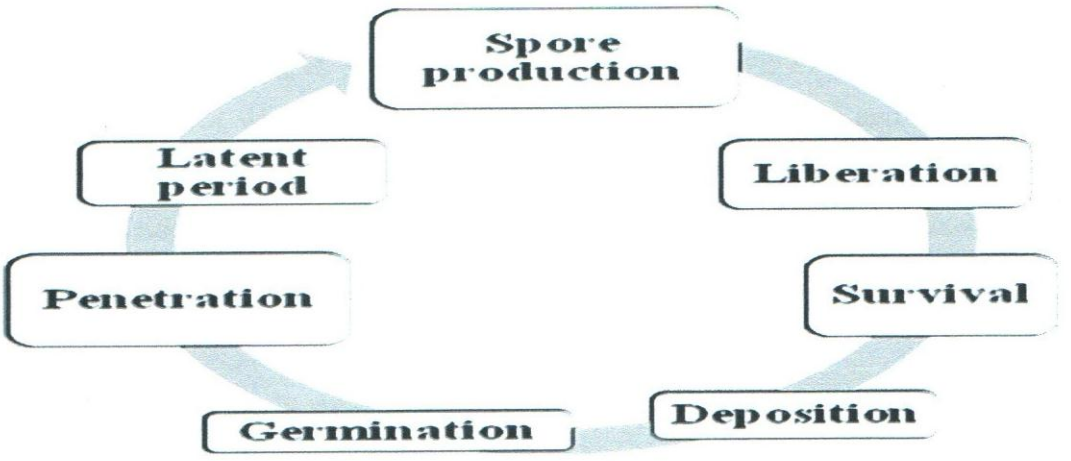


Figure 2a: Polycyclic events characterizing an epidemic in the field (After Teng *et al.*, 1977)

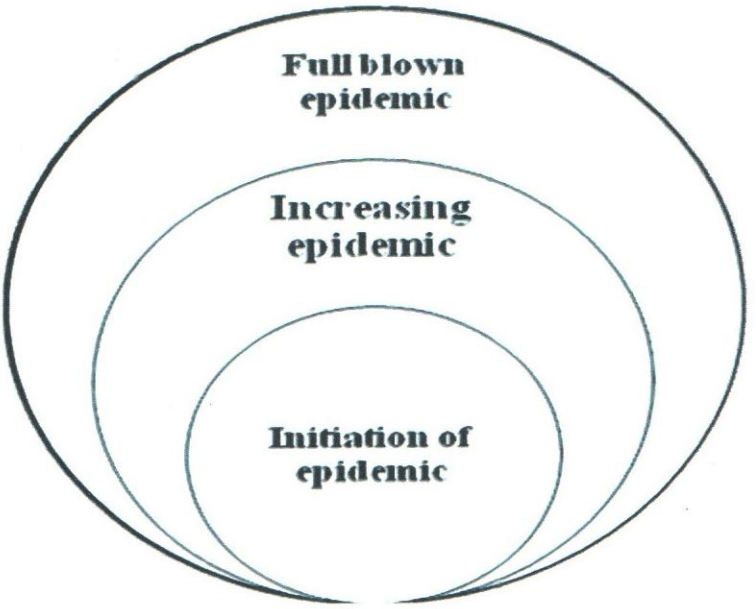


Figure 2b: Steps through distinct rust monocycles (After Teng et al., 1977)

A rust epidemic occurring in the field (polycycle) can be thought of as resulting from a series of monocycles, each of which in turn comprises distinctive processes and the whole of which is integrated into disease progress curve to describe the epidemic (Figure 2a) (Teng *et al.,* 1977). In a stem rust epidemic component processes of the rust monocycle recognized are as shown in Figure 2b.

2.1.3 Genetic variation in stem rust of wheat pathogen

The distribution and frequency of pathogen genes and genotypes in a particular area is a function of several factors. These are:

Mutation. Mutations are the most likely and most predictable events that contribute to short-term rust pathogen variability (McIntosh, 1988). Genes for virulence in the rust pathogens are generally recessive. Recessive mutations occur much more frequently than dominant ones,

although their frequency is low, perhaps of the order of $1x10^{-5}$ or 10^{-6}. Since the rusts are dikaryotic, a double mutant will be needed.

Recombination. The sexual cycles of rust pathogens have obvious implications both for the evolution of new pathotypes and for seasonal carry-over of inoculum (McIntosh, 1988). For stem rust and leaf rust, the sexual cycle can be an important source of new combinations of genes of virulence wherever the alternate host occurs.

Somatic hybridization. In many fungi, variation can result from the fusion of hyphae from different pathogen genotypes, followed by nuclear exchange and the reassortment of different nuclei in binucleate or multinucleate cells. Nelson *et al.* (1955) observed the fusion of hyphae from different stem rust biotypes on a thin agar film.

Selection pressure. The genotypes of the predominant wheat commercial cultivars as well as those of barley, and other grasses on which rust survives between seasons will influence the pathogen genotypes that survive.

2.2 Race identification and naming in *Puccinia graminis* f.sp. *tritici*

The discovery of race 1 and 2 by Stakman and Piemeisal (1917) lead to what is now designated as physiologic races of *Puccinia graminis*. The physiologic race of 1922 was based on the 12 differential host cultivars; Little club, Marquis, Kanred (later replaced by Reliance), Kota, Arnautka, Mindum, Marz (known now as Spelmer), Kubaka, Acme, Einkorn, White spring emmer (now called Vernal), and Khalpi. Infection types were divided into groups, with 0, 1, and 2 indicating host resistance and infection types 3 and 4 indicating susceptibility. The X infection type was considered separately as a heterogeneous host response. Physiological races were determined by a dichotomous key of resistant and susceptible host responses with the heterogenous responses in a separate section of the key. The differential hosts selected by Stakman and co-workers in 1917 were used worldwide until the mid-1960s through the earlier years of the 80s (Roelfs and Matens, 1988). The changes in the classification of *P. graminis.* f. sp. *tritici* resulted from two major scientific events; the development of the gene-for-gene concept, principally by Flor (Flor, 1971) and the development of host lines differing primarily by a single effective gene of resistance. The earliest lines that contained single genes for resistance

to wheat stem rust were developed by Knott, Anderson, Watson and Luig, and Loegering and Harmon (Roelfs and Matens, 1988).

The acceptance of single-gene differentials for race identification came rapidly, involving different systems of nomenclature, i.e., Australia in 1963 (Watson and Luig, 1963), Canada in 1965 (Knott and Anderson, 1956) and the United States in 1972 (Roelfs and McVey, 1979). A method that has gained wide application is that developed and improved at the Cereal Rust Laboratory, St. Paul, MN (Jin *et al.*, 2008). In this case, differential lines that carry single genes *Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9d, Sr9e,* and *Sr15* in Chinese spring background and *Sr9b, Sr10, Sr13, Sr15*, and *Sr36* in the background of W2691, a highly susceptible line developed at the University of Sydney, Vernstein *(Sr9e)*, combination VII *(Sr13 and Sr17)* and Triumph *(Sr Tmp)* are employed as testers. To designate a race, a coding system is employed. Twenty of the testers are divided into five sets of four lines (Table 2).

Table 2: The 20 North American differential wheat lines for differentiating races of *Puccinia graminis* f. sp. *tritici* in ordered sets of four

	Stem rust genes in the wheat differentials			
Host set 1	5	21	*9e*	*7b*
Host set 2	11	6	*8a*	*9g*
Host set 3	36	*9b*	30	17/13
Host set 4	*9a*	*9d*	10	*Tmp*
Host set 5	24	31	38	*McN*

(Jin *et al.*, 2008)

The first set includes the genes *Sr5, Sr21, Sr9e,* and *Sr7b.* The other four sets include some of the more recently identified genes of race identification. When four lines are classified for resistance (IT 0-2) or susceptibility (IT 3-4) (Figure 3 and Table 4) there are 20 possible combinations (Table 3). These are coded from B to T, omitting the vowels. The pathogenicity of a race is coded using five letters, each indicating its pathogenicity on one set of four lines. Code BBBBB

would indicate that all the tester lines are resistant while TTTTT would indicate that all 20 are susceptible.

Table 3: Code (*Pgt*) for the 20 North American differential hosts

***Pgt* code**	**Infection Response**			
B	Resistant	Resistant	Resistant	Resistant
C	Resistant	Resistant	Resistant	Susceptible
D	Resistant	Resistant	Susceptible	Resistant
F	Resistant	Resistant	Susceptible	Susceptible
G	Resistant	Susceptible	Resistant	Resistant
H	Resistant	Susceptible	Resistant	Susceptible
J	Resistant	Susceptible	Susceptible	Resistant
K	Resistant	Susceptible	Susceptible	Susceptible
L	Resistant	Susceptible	Susceptible	Susceptible
M	Resistant	Susceptible	Resistant	Susceptible
N	Susceptible	Resistant	Susceptible	Resistant
P	Susceptible	Resistant	Susceptible	Susceptible
Q	Susceptible	Susceptible	Resistant	Resistant
R	Susceptible	Susceptible	Resistant	Susceptible
S	Susceptible	Susceptible	Susceptible	Resistant
T	Susceptible	Susceptible	Susceptible	Susceptible

(Jin *et al.*, 2008)

2.3 Symptomatology

Infections in wheat occur mainly on stems and leaf sheaths, but occasionally they may be found on leaf blades and glumes as well. The first macroscopic symptom is usually a small chlorotic fleck, which appears a few days after infection. About 8-10 days after infection, a pustule several millimeters long and a few millimeters wide is formed by rupture of the host epidermis from a mass of brick-red urediospores produced in the infection (Leonard, 2001).

These pustules are generally linear or diamond shaped and may enlarge up to 10mm long. The powdery masses of urediospores appear similar to rust spots on weathered iron surface (Kurt and

Szabo, 2005). With age the infection ceases production of brick- red urediospores and produces a layer of black teliospores in their place, causing the stems of heavily infected plants to appear blackened late in the season.

2.4 Stem rust rating system

Infection types for uredinia on seedling leaves are commonly designated based on a scale developed by Stakman *et al.* (1962). This gives infection types as 0, 1, 2, 3 or 4 with an extra class designated as X for heterogenous or mesothetic infections (Stakman *et al.*, 1962) (Figure 3, Table 4). In practice, infection types form a continuum and there is no clear-cut demarcation between types. Four convenience infection types, 0, 0, ;, 1 and 2 are considered resistant while 3 and 4 are considered susceptible.

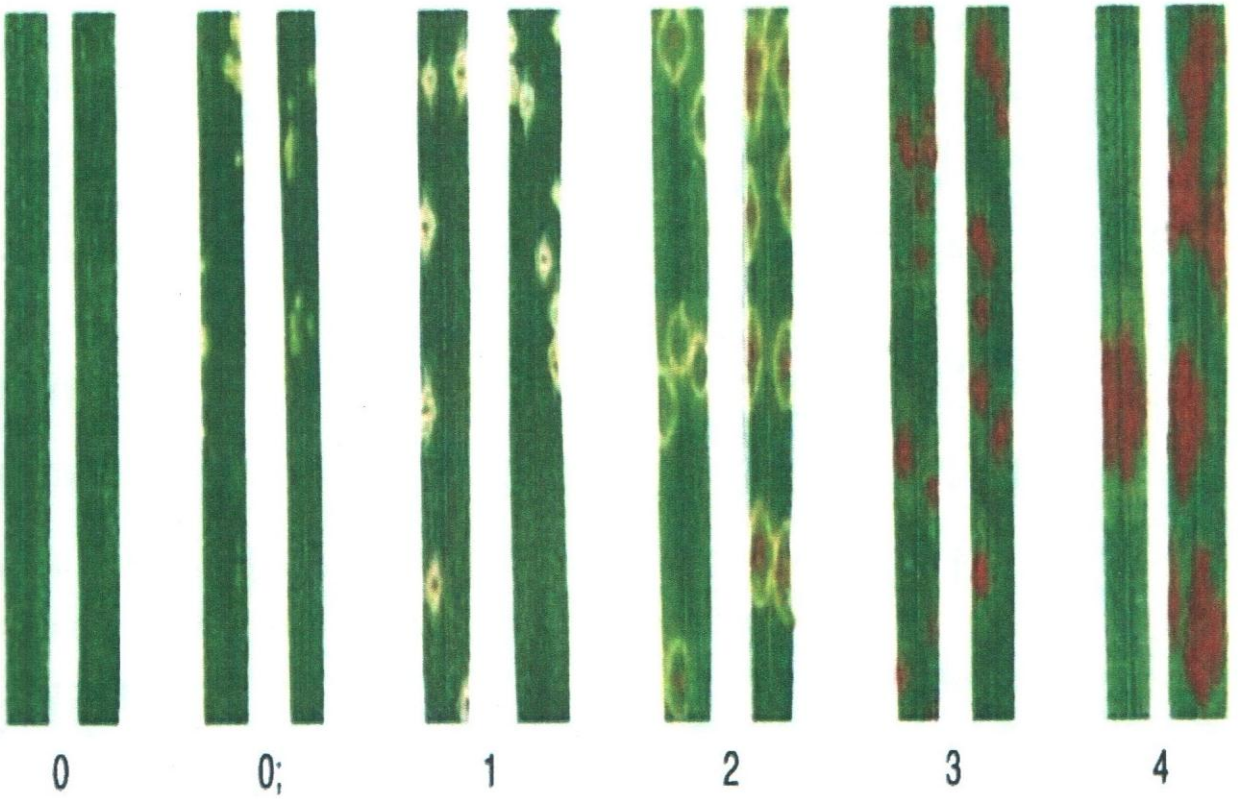


Figure 3: Infection types of wheat stem rust (Leonard and Szabo, 2005)

Table 4: Description of infection types used in classifying the reaction to stem rust on seedling wheat leaves on a scale of 0-4

Infection type	Description	General Reaction
0	No uredinia or other macroscopic signs of infection	Resistant
0;	No uredinia, but hypersensitive necrotic or chlorotic flecks of varying size present	Resistant
1	Small uredinia often surrounded by chlorosis or necrosis	Resistant
2	Small to medium uredinia often surrounded by chlorosis or necrosis	Resistant
3	Medium sized uredinia without chlorosis or necrosis	Susceptible
4	Large uredinia without chlorosis or necrosis	Susceptible

(Adapted from Stakman *et al.,* 1962)

2.5 Management of stem rust of wheat

2.5.1 Cultural methods

Cultural practices are primarily aimed at breaking the life cycle of the rust. Early infection sometimes arises from volunteer plants and delayed planting may help. In Kenya wheat is grown all year round and this leads to a green bridge that helps to carry the spores from one season to the other. Therefore, cultivation to remove volunteer plants and trying to avoid green bridge may also reduce sources of inoculum (Zadoks and Bouwman, 1985). Alternate host eradication has been successfully carried out in Northern Europe (Hermansen, 1968) and North Central States of USA (Roelfs, 1978) with great success. Barberry bushes, if they occur, should be eradicated since they act as an intermediate host. In Australia early maturing varieties have been successfully used in controlling stem rust (McIntosh, 1976) and in Mexico farmers used to plant

early to escape wheat stem rust epidemics before resistant varieties were introduced (Borlaug, 1954).

2.5.2 Genetic control

Development of resistant cultivars is the most effective method of control of rusts (Johnson, 1992). Their use adds no extra cost to farmers. Breeding for resistance in many wheat growing countries is a never-ending task that goes through a repeating cycle. Wheat breeders are increasingly interested in types of resistance and methods of using resistance that will result in long-lasting control of the rusts, also termed durable resistance (Bruce *et al.,* 2002). Some of the stem rust resistant *(Sr)* genes have been widely deployed in commercial wheat cultivars. However most of them are susceptible to race Ug99.

Sr2, which has a slow rusting resistance gene, has been used in some local varieties like Kwale but this variety does not have enough adult plant resistance against race Ug99 (Njau *et al.,* 2009). *Sr24* has been a good source of resistance against race Ug99 until 2007 (Singh *et al.,* 2007; Jin *et al.,* 2008) when farmers started experiencing epidemics on variety Mwamba which is known to carry *Sr24.* The effectiveness of *Sr24* to the new virulent isolates of Ug99 has been decreased (Jin *et al.,* 2008). *Sr31* has for long been used as a source of resistance against all races of wheat stem rust but detection of race Ug99 (Pretoruis *et al.*, 2000) and subsequent screening against our local varieties has proved the virulence of race Ug99 against this race (Macharia, 2009; Njau *et al.,* 2009). *Sr36* has been showing high to medium resistance to the original race Ug99 and has been successfully used in Africa to control this race (Singh *et al.*, 2007). Recent detection of new virulent isolates of race Ug99, TTTSK, that gives a high infection type on *Sr36,* renders the *Sr36* ineffective. Therefore, *Sr36* is no longer recommended for use as useful source of stem rust resistance against Ug99 (Jin *et al.,* 2009). The new race of Ug99 has overcome many known *Sr* genes. Genes *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9g*, *Sr10*, *Sr11*, *Sr12*, *Sr15*, *Sr16*, *Sr17*, *Sr18*, *Sr19*, *Sr20*, *Sr23*, *Sr30*, *Sr31*, *Sr34*, *Sr38* and *SrWld-1* (Jin *et al.*, 2007) were all defeated by the Ug99 (Pretoruis *et al.*, 2000; Jin and Singh, 2006).

Currently only a few known genes are still effective against Ug99, and they include *SrTm* which results in low infection type to race Ug99 (Singh *et al.,* 2007). *Sr1A/1R* derived from *Secale cereale* provides resistant to race Ug99 and has been detected in several hard red winter wheat cultivars (Sebesta *et al.,* 1995; Jin and Singh, 2006). Other genes, including *Sr22*, *Sr25*, *Sr35*, *Sr39*, *Sr40* and *Sr44* identified from different wheat relatives, are moderately to highly resistant to race Ug99 in seedling tests (Jin *et al.,* 2007; Singh *et al.,* 2007). Although these genes have shown resistance to race Ug99 their use is uncertain considering the ever mutating of race Ug99 as reported by Jin *et al.* (2008; 2009).

2.5.3 Chemical control

While resistance is the most effective method of controlling stem rust, there are no commercial varieties in Kenya with adequate resistance (Njau *et al.*, 2009). Therefore, fungicides as foliar or seed treatments will play a role in the integrated management of the disease until new varieties with improved resistance are released. The disease buildup has increased over the years that in the year 2007, epidemic levels were observed in farmers' fields (Wanyera, 2008a). Most fungicides by then had been developed for yellow rust *(Puccina striiformis)* and leaf rusts *(Puccinia recondita)*. Effective control of stem rust can be achieved with standard application of fungicides (Wanyera *et al.,* 2009). There is therefore urgent need to develop economic spray routines that a farmer can adopt for some of the stem rust recommended fungicides, which are Amister Xtra 280SC, Folicur 250EC, Silvacur 375EC and Oriou 25EC.

CHAPTER THREE

WHEAT STEM RUST SEVERITY AND PHYSIOLOGICAL RACES IN UASIN GISHU REGION

ABSTRACT

To effectively utilize stem rust resistance, it is necessary to monitor the virulence composition and dynamics in the stem rust pathogen population, which provides a continuous threat to released varieties and breeding materials. Timely identification of a race will assist breeders to develop a resistant variety in time before an epidemic. A study was therefore carried out in Uasin Gishu region of Kenya to determine the severity of wheat stem rust and identify the physiological races of *Puccinia graminis* f.sp *tritici*. A stratified random sampling design was used, where the region was divided into five stratas based on wheat growing areas. A Global Positioning System (GPS) was used to get the geographical data of the sampling sites. Wheat stem rust severity was determined using the modified cobs scale of 0-100% (Peterson *et al.*, 1972), while stem rust races were identified using the 20 northern American wheat differentials. The results revealed a significant *($P \leq 0.05$)* variation in wheat stem rust severity in the region. Lower wheat stem rust severity of 12.5% was observed in high altitude areas (>2200 m.a.s.l) of Kipkabus. High wheat stem rust severity of 34% and 32% was observed in lower altitude areas (<1900 m.a.s.l) of Mois Bridge and Moiben respectively. Four wheat stem rust races were reported in this region. The predominant race was TTKST *(Ug99+Sr24)* at 66.7% predominance, followed by race TTKSK *(Ug99)* at 21.1%, then race PTKST *(Sr31+Sr24-Sr21)* at 10.5% and race TTTSK *(Ug99+Sr36)* at 1.8%. A new race PTKST was reported at Chekoilel, Moiben and Mois bridge. There was no significant correlation (r=0.398) of stem rust severity and race diversity. However, there was a significant ($P \leq 0.05$) negative correlation (r=-0.461) of race diversity and altitude and a highly significant ($P \leq 0.01$) negative correlation (r=-0.713) of altitude and stem rust severity. The study concluded that wheat stem rust severity varied depending on altitude and location, four races are present in Uasin Gishu region and the predominant race is TTKST, *(Ug99+Sr24)*. A new race PTKST *(Ug99+Sr24-Sr21)* was reported and is also widespread. The study therefore recommended a continued survey to other wheat growing regions of Kenya to determine the distribution of the races in the country.

3.1 Introduction

Wheat is planted in Kenya to maximize use of rainfall while the crop is growing, yet have it ready for harvest during the dry period. In most of Kenya's wheat growing areas ranging from 1800-2800m meters above sea level (Payne *et al.,* 2000), the long rains usually occur between March and August, the short rains during October and November. Dry conditions usually prevail from December to March (Green *et al.,* 1968). Most wheat is planted from March to June and harvested from August to January. Only a small proportion of the crop is grown above 2400m and on a small acreage. On this land rust inoculum can be produced, and remains standing in January and February as a reservoir for rust in the main wheat growing season. Urediospores produced on this wheat could be in the air after the new crop at lower altitudes has emerged. The wheat growing season has temperature characteristically ranging from 18-30°C with days uniformly about 12.5 hr long. Dews are heavy and precipitation occurs frequently as showers during the main growing season. All these factors make wheat stem rust prevalence in Kenya high, to evolution of new races. Uasin Gishu region in Kenya is one of the leading wheat producing areas. The region has a wide agro ecological zones and altitude ranges from 1800m above sea level at Mois bridge area to more than 2700m above sea level at Kipkabus area. The region is considered the grain basket of Kenya and about 37,000 ha of this area is covered by wheat which represents about 25% of the total wheat grain production in Kenya (70,000 – 90,000 tons annually) (MoA, 2010). As part of the countrywide race identification the region accounts for a significant portion of the surveys. For these reasons an experiment was conducted to determine the distribution of stem rust races, and to characterize stem rust spore collections from different sites in Uasin Gishu region.

3.2 Literature Review

Previous work on race identification has shown that races of wheat stem rust change from year to year. Roelf, (1980) carried out work between1973-1980 and the results showed variations on the number of virulent races each year where the most predominant race during this period was found to be race TNM. In 1995 67% of the isolates from a farm in USA were found to be race TPMK, other races identified were race QCCJ, QCCS, QCMS, QFCS, and TPMK (Mc Vey *et*

al., 1997). In 1996 race TPMK represented 76% of the isolates from the field, race QFCS represented 12% of the isolates while eight other races consisted 3% or less of the isolates (Mc Vey *et al.,* 1999). In 1997 race TPMK represented 69% while race RCRS represented 11%. This however changed and by 1998 race TPMK was 10% and race RCRS was 55% (Mc Vey *et al.,* 2002). Recent works in USA reported two new races i.e. race QFCN and race TTTT, (Jin, 2005) and the most predominant race was QFCS. In Ethiopia Belayneh and Emebet (2005) reported a total of 44 different races in a period of five years. The races differed greatly from year to year and race TTR was the only race identified in all cropping seasons. Other races identified were race TTT, PTT, PTR and TTQ. During the period 2006/2007 a total of 22 races were reported (Belayneh *et al.,* 2008) and race TTKSR *(Ug99),* TTHSR and RRTTR were the most predominant during this period, similar work has also been reported in Canada (Fetch and Dunsmore, 2004).

In Kenya two stem rust physiological forms were differentiated in 1927 (Green *et al.,* 1968). Later another form appeared on wheat variety *Reliance* at Njoro in 1930 and a forth one was recognized in 1933. By 1947, eight stem rust races (on the basis of Kenya differentials) had been identified (Payne *et al.,* 2000). Four more races appeared three years later with little or no stable resistance available in the Kenyan genepool. Based on international race differentiation system, the races *K1, K5* and *K12* were identified as American 'race 21' and *K8, K10* and *K11* as American race 24 (Thorpe, 1958). For the last thirty years little work on race identification of stem rust has been going on, more because most varieties released had *Sr 31* which was effective against all the previous identified stem rust races. With the breakdown of *Sr 31* gene in wheat (Pretoureus *et al.,* 2000), new races are emerging. Currently three new races have been reported, race TTKSK (Wanyera *et al.,* 2006), race TTKST (Jin *et al.,* 2008) and race TTTSK (Jin *et al.,* 2009). These races were identified from varieties/lines which were known to carry resistant genes but a detailed survey has not yet been carried out to study the extent of these races or even any other new races in Kenya. Race surveys helps to generate information regarding the virulence of races and their frequency and distribution patterns across regions and over time. This is of paramount importance in developing wheat cultivars with durable stem rust resistance. In addition, virulence surveys are important for studying the evolution of new races and forecasting the virulence shifts in a population. This information helps breeders to develop a

strategy and to respond to sudden changes in virulence, thereby preventing a resistance breakdown in wheat cultivars.

3.3 Materials and Methods

3.3.1 Determination of wheat stem rust severity and distribution

A survey was carried out when the wheat crop was between GS 60- GS 85 (flowering-soft dough stage) (Zadok *et al.*, 1974). The survey was carried out using the stratified simple random sampling technique (Deng *et al.,* 2009) where the strata represented the five zones in Uasin Gishu region where wheat is grown most and were considered to be homogenous sampling blocks i.e. Northern strata (Moiben), Southern strata (Mosoriot), Western strata (Kipkabus), Central strata (Chepkoilel/Sergoit) and Eastern strata (Mois Bridge).

A total of 360 km was covered, and the procedure described by Park, (2008) for wheat rust sampling was followed, where sampling sites were identified in the farmers' fields by following both main roads and feeder roads. A sample was taken after every 20 km (Jin, 2005), and the number of sampling sites within a strata depended on the area covered by wheat within that strata. Once a sampling site was identified the geographic data (latitude, longitude and altitude) were recorded using a GPS (e Trex Legend GPS System, Garmin). Wheat stem rust severity was determined by walking into the field beyond the borders and then examining approximately 33 m across the field as described by Roelf *et al.* (1992). Stem rust severity was scored using the modified cobs scale of 0-100% (Figure 4), where 0= no rust infection and 100%= complete cover by rust uredia both on leaves and stem (Peterson *et al.,* 1972). Severity at different sites was analyzed using descriptive statistics (frequencies and percentages) and direct correlation, using the statistical package for social scientists (SPSS 17) computer software.

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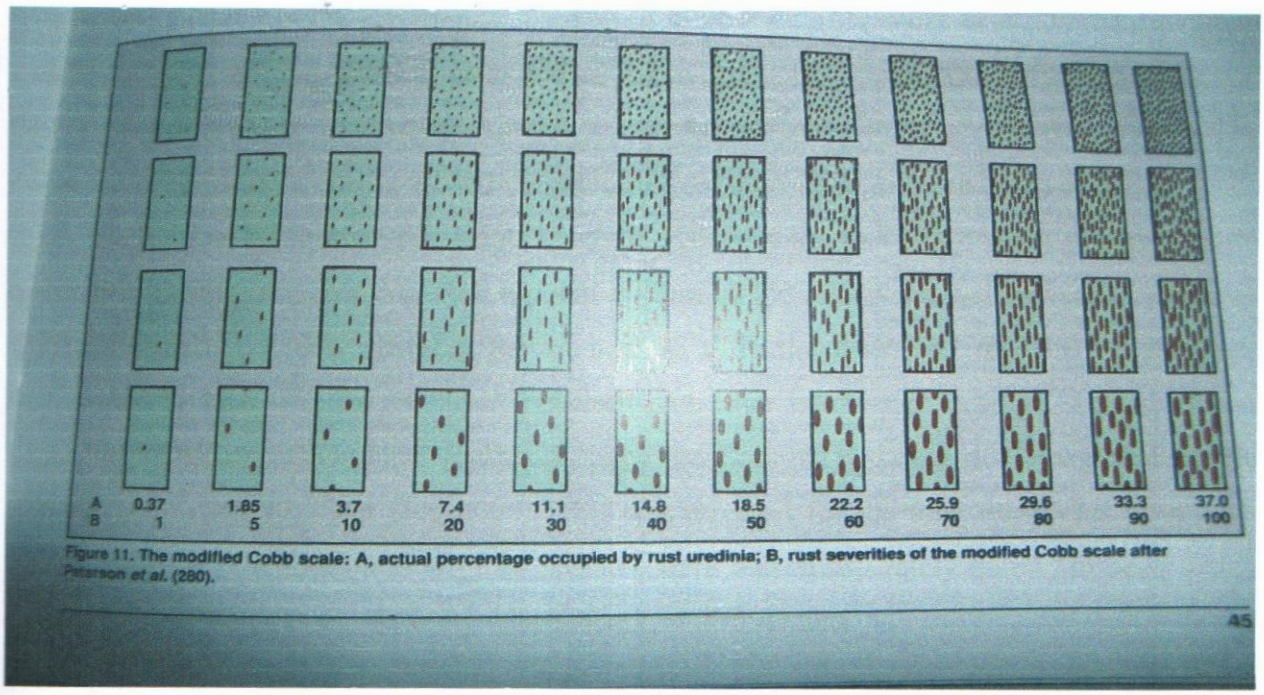


Figure 11. The modified Cobb scale: A, actual percentage occupied by rust uredinia; B, rust severities of the modified Cobb scale after Peterson *et al.* (280).

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3.3.2.1 Samples collection

Following the survey described in 3.3.1 above, from each sampling site where wheat stem rust severity was determined, about fifty samples (5 cm stems of wheat that had susceptible pustules) were collected by cutting them using a sterilized pair of scissors. A cooling box was used to preserve the samples while in transit to the laboratory. Later the samples were air dried for 12 hours before storing them in a refrigerator at 3-5°C (Green, 1981) awaiting inoculation.

3.3.2.2 Wheat stem rust differentials used

Twenty Northern America wheat differential hosts *(Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 30, 17(+13), 9a, 9d, 10, Tmp, 24, 31, 38* and *McN.)* were used to characterize the isolates of *Puccinia graminis* f.sp. *tritici* (Jin *et al.*, 2008).

3.3.2.3 Multiplication and isolation of stem rust spores

Cv Morocco (a universal susceptible genotype to stem rust) was planted for spore isolation and subsequent bulking. Three plants of seven day old *cv Morocco* were inoculated with spores collected from each site/field. This was repeated for all sites on different *Morocco* seedlings

which were planted in the greenhouse maintained at 26-30°C (McIntosh *et al.,* 1995). Inoculum was prepared by mixing distilled water and oil tween-20 at one drop/500ml of distilled water and then suspending 2 mg of urediniospores/1 ml of the mixture (Welty and Barker, 1992). The suspension was sprayed using a hand operated sprayer onto 7-day-old seedlings of the *Morocco* lines. Each set of three *Morocco* plants received 100ml of the inoculum which was sprayed to cover the whole plant ensuring both top and bottom of the leaves are well covered. Seedlings were incubated in a dew chamber for 14 h at 18°C in the dark, and then in a greenhouse maintained at 18 ± 2°C for 14 days. Fourteen days after inoculation pustules were separated according to their sizes/virulence pattern of; large, small, round and ragged. Three pustules were derived from each site/field (Jin, 2005). This was repeated for the four sites/fields from each zone. From the five zones a total of 60 pustules were bulked separately on *cv Morocco* which were later tested on the set of twenty wheat stem rust differentials.

3.3.2.4 Race identification

The twenty differential hosts were seeded in plastic pots filled with a mixture of sterile silt clay, sand and forest soil. Each pot contained four differential genotypes in the following order (i) *Sr5, 21, 9e, 7b*; (ii) *Sr11, 6, 8a, 9g*; (iii) Sr*36, 9b, 30, 17*; (iv) *Sr 9a, 9d, 10, Tmp;* (v) *Sr24, 31, 38* and *McN* (Jin *et al.,* 2008). The bulked single-pustule isolate derived from each collection was inoculated by mixing distilled water and oil tween-20 at one drop/500ml of distilled water and then suspending 2 mg of urediniospores/1 ml of water and oil tween-20 (Welty and Barker, 1992). The mixture was stirred vigorously using a spatula, while diluting with distilled water. The suspension was sprayed using a hand operated sprayer onto 7-day-old seedlings of the differential lines. Each set of four differentials received 100ml of the inoculum which was sprayed to cover the whole plant ensuring both top and bottom of the leaves are well covered.

Seedlings were incubated in a dew chamber for 14 h at 18°C in the dark, and then in a greenhouse maintained at 18 ± 2°C for 14 days. Infection types were scored (Stakman *et al.,* 1962) on designated *Sr* genes, 14 days after inoculation using **0-4** infection type (IT) scale (McIntosh *et al.*, 1995) when the uredia on the susceptible variety *Morocco*, which acted as a control appeared fully developed. A susceptible host response characterized by large uredia

without chlorosis or necrosis was denoted by **"4"**, whereas no visible uredia, flecking and small uredia with necrosis was denoted by **"0"** and **"1"** respectively. Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis and medium sized uredia with or without chlorosis was indicated by **"2"** and **"3"** respectively. Variations with less or more than the average of a class was indicated by the use of superscript **"+"** for pustules that are larger or **"-"** for pustules that are smaller than the normal size limit. Each isolate was assigned a five-letter race name based on its reaction on the differential lines. The experiment was repeated twice, and only differential hosts that produced similar infection types in the two experiments were considered. For infection type 0 (immune reaction), the test was repeated to exclude the possibility of disease escape.

3.4 Results and discussion

3.4.1 Wheat stem rust severity and distribution

The general visual disease severity scores on locally grown wheat crop, made from the five zones (i.e., Chepkoilel/Sergoit; Mois' Bridge; Moiben; Kipkabus and Mosoriot) are given in Table 5 below. Stem rust disease severity of 33 and 34% on locally grown wheat was observed to be significantly ($P \leq 0.05$) higher in Moiben and Mois' Bridge area, respectively. Mois' Bridge reported the highest stem rust severity of 34%, with an altitude range of between 1714-1946 m.a.s.l. The highly significant ($P \leq 0.01$) negative correlation ($r=-0.461$) of stem rust severity and altitude, indicates that the high stem rust severity recorded at this zone could be attributed to the zone having the lowest altitudes. The low altitude offers high temperatures and during the cropping season there is high humidity which provide more conducive conditions for infection, spread and growth of wheat stem rust (Roelf *et al.*, 1992).

Table 5: Wheat stem rust severity in Uasin Gishu region at different sites during the May-Nov 2009 cropping season

Zone	Mean stem rust severity (%)
Chepkoilel	23±3.3
Mois' Bridge	34±2.5
Moiben	33±2.5
Kipkabus	13±2.5
Mosoriot	13±3.3
Total	24±2.5

Means of wheat stem rust severity whose SE overlaps are not significantly different at 0.05 level of significance.

Moiben area recorded a mean stem rust severity of 33% which was found to be not significantly ($P \leq 0.05$) different from that of Mois' Bridge. Moiben is located at slightly higher altitude range of between 1944-2150 m.a.s.l. The region is associated with very large fields of monocropping (wheat and barley) and the proximity of this zone to Mois' Bridge, which had the highest stem rust severity, could explain why this region too had a high stem rust severity. Chepkoilel/Sergoit recorded a mean stem rust severity of 23% which was found to be significantly ($P \leq 0.05$) lower than that recorded at both Mois' Bridge and Moiben zones but significantly higher than that recorded at both Kipkabus and Mosoriot. With an altitude range of between 2144-2147 m.a.s.l, high stem rust severity would have been expected as that reported at Moiben. However a lower severity of about 23% was recorded, which was mainly attributed to chemical control (Tebuconazole 250g/L at 1 l/ha applied twice during the crops cycle) which had been used at two of the three farms surveyed (Rop, Pers comm.). Both Kipkabus and Mosoriot area recorded significantly ($P \leq 0.05$) low stem rust severity than all the other zones. Kipkabus area which is located at altitudes of between 2595-2718 m.a.s.l recorded the least mean stem rust severity of 13%, with a highly significant ($P \leq 0.01$) negative correlation between altitude and stem rust severity. The high altitude of this area can perhaps explain why low stem rust severity was recorded. More so most farmers in this area are small scale practicing mixed cropping and this

could also contribute to the low stem rust severity reported. Although Mosoriot area is located at altitudes favorable for rust epidemics i.e. 1996-2134 m.a.s.l, a low severity of 13% was recorded. The area is geographically isolated to the other four wheat growing zones, more so most farmers in this area were small scale and mixed cropping was common. This could perhaps explain why the area recorded a low stem rust severity.

3.4.2 Wheat stem rust races

Stem rust races identified from the 57 isolates derived from the samples collected from all the five zones of Uasin Gishu region are given in Table 6. Chepkoilel recorded the highest race diversity, with the nine pustules isolated yielding four races; where two pustules yielded race TTKSK; four were of race TTKST; one of TTTSK and two of PTKST. The high race diversity including the new race PTKST is not unusual, since two of the three fields where samples were taken are mostly used for research purposes and thus different wheat materials/varieties are planted and this could be the reason why there was a high race diversity. Similarly, previous studies have shown that the type of race identified in a place greatly depends on the cultivars planted (Belayneh *et al.,* 2008). The gene for gene theory dictates that for a virulent race to be present, there must be a resistant gene against that pathogen (Flor, 1971). At Mois' Bridge, out of the 12 pustules tested, three stem rust races were identified. These were one pustule of TTKSK; nine of TTKST and two of PTKST. Race TTTSK was not identified in this zone.

Table 6: Wheat stem rust races in Uasin Gishu region at different sites during the May-Nov 2009 cropping season

Site	Source	Collection	Isolate	Number of isolates of *Pgt* races			
				TTKSK	TTKST	TTTSK	PTKST
Chepkoilel/Sergoit	Nursery and Field	3	9	2	4	1	2
Mois' Bridge	Field	4	12	1	9	0	2
Moiben	Field	5	15	2	11	0	2
Kipkabus	Field	4	12	4	8	0	0
Mosoriot	Field	3	9	3	6	0	0
TOTAL		19	57	12	33	1	6
TOTAL %				21.1	66.7	1.8	10.5

The predominance of race TTKST in this zone i.e. nine pustules out of 12 tested can perhaps be attributed to the commonly grown variety in this zone, the variety *KS Mwamba* which is known to carry *Sr 24*, which this race is virulent to (Njau *et al.*, 2009). The new race PTKST was also detected in this zone and since this race is virulent to *Sr 24* gene too, perhaps explaining its presence. At Moiben, out of the 15 pustules tested, three races were identified. These were two of TTKSK; 11 of TTKST and two of PTKST. Race TTTSK was not identified in this zone and race TTKST was the most predominant in this zone i.e. 11 of the 15 pustules tested. Similarly the new race PTKST was also reported in this zone. At Kipkabus, of the 12 pustules tested only two races were identified. These were four of TTKSK and eight of TTKST. In this zone the new race PTKST as well as race TTTSK were not identified. The data (Table 7) shows that there was a significant ($P \leq 0.05$) negative correlation ($r=-0.713$) on number of races identified with altitude, thus the two races identified at this site could be attributed to the area being located at high altitudes. At Mosoriot, of the nine pustules tested only two races were identified. These were three of TTKSK and six of TTKST. Just like Kipkabus, this zone did not report the new race PTKST and also race TTTSK. Although the zone is located at slightly lower altitude where stem

rust races diversity is expected, the low stem rust severity of 13.33% reported could be the reason why there were fewer races. Similarly the zone is geographically isolated from the other four, perhaps explaining the low race diversity.

It should be noted that race TTTKS was only identified at Chepkoilel site whereas the new race PTKST, was reported at three sites i.e. Chepkoilel, Mois' Bridge and Moiben. Perhaps the proximity to these areas to each other, with no host barrier can explain this. Of the four races identified in Uasin Gishu region race TTKST had 70% predominance, the widespread cultivation of variety *KS Mwamba* which is known to carry *Sr* 24 (Njau *et al.,* 2009) can explain perhaps why race TTKST is predominant in this region. This was followed by race TTKSK (15%), PTKST (11.5%) and TTTKS (3.3%) respectively.

The study confirmed the widespread distribution of wheat stem rust in Uasin Gishu region. Wanyera *et al.* (2006) reported race Ug99 all over Kenya. However from the present study, there appear to be change in virulence dynamics within *pgt* populations. A mutant of race Ug99 (TTKST) which was detected at Njoro in 2008 (Jin *et al.,* 2008) appears to be the most predominant race in this region. The change in virulence dynamics over time is not new as this has been reported in other places like U.S.A (Jin, 2005) and Ethiopia (Belayneh *et al.,* 2008). From the survey, it was confirmed that most farmers in this area had planted variety *KS Mwamba* and since this variety is known to carry *Sr 24* gene, the predominance of race TTKST *(Ug99+sr 24)* is possible. Although it took many years for race Ug99 to cause epidemics on farmers' fields from when it was reported in Uganda (Pretoureus *et al.,* 2000) up to 2005 when epidemics were reported (Wanyera *et al.,* 2006), this race appear to be even more virulent as it was only reported in 2007 at KARI Njoro Kenya (Jin *et al.,* 2008) and is currently causing epidemics in Uasin Gishu region.

Identification of a new race PTKST with a predominance percentage of 10.5% is significant. Considering this race is also virulent to *Sr 24* gene which is present in *KS Mwamba* and its widespread cultivation in this area, perhaps it is only a matter of time before this race becomes widespread in this region. Whether this race is a mutant from the existing races or an introduction, currently remains unknown. However, its detection at Moiben, Mois Bridge and Chepkoilel, where high stem rust severity was reported and the three areas being in close

proximity to one another and its absence in higher altitudes of Kipkabus and isolated areas of Mosoriot, could be an indication that it could be an introduction either through Chepkoilel or a mutation caused by high inoculum pressure as suggested by Sign *et al.* (2006) and Jin *et al.* (2008). Latest studies have also confirmed race PTKST in South Africa (Pretourius *et al.*, 2010) where it is claimed to have been introduced from unknown sources. Though the original Ug99 race is virulent, recent studies by Pretorius *et al.* (2010) have pointed out the importance of the mutants of this race, which are being predicted to even be more virulent on the previously resistant materials.

3.4.3 Correlation of race diversity, stem rust severity and altitude

Correlation was performed using MSTATC statistical package and the data (Table 7) revealed that, there was no significant correlation between race diversity and stem rust severity, which implies that the number of wheat stem rust races identified in a place is not related to wheat stem rust severity. Therefore, it is possible to have a few races with a very high rust severity and vice versa.

Table 7: Correlation relationship between race diversity, stem rust severity and altitude

	Race diversity	**Stem rust severity**	**Altitude**
Race diversity	1	0.398	-0.461*
Stem rust severity		1	-0.713**
Altitude			1

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Similar studies as reported by Wanyera *et al.* (2006) showed race Ug99 (TTKS) alone in farmers' fields caused up to 70% severity in Kenya. Similarly Belayneh *et al.* (2008) in Ethiopia reported up to 12 races with a severity of 27% only at Shewa area, while Jin (2005) reported five races from fields with trace amounts (<10%) of wheat stem rust severity in U.S.A. There was a

significant ($P \leq 0.05$) negative correlation of $r=-0.461$ on race diversity with altitude. This implies that the number of races is likely to increase as altitude reduces. However it is important to note that altitude alone might not be the cause as previous work has shown that race diversity is also dependent on varieties planted and cultural practices employed as well as the environmental conditions (Singh, 1991; Roelf *et al.*, 1992). Perhaps the low altitude favors the environmental conditions necessary for evolution and survival of wheat stem rust races. There was a highly significant ($P \leq 0.01$) negative correlation of $r=-0.713$ on wheat stem rust severity and altitude. This implies that stem rust is more likely to be severe in low altitude areas than in higher altitude areas. Low altitude favors high temperatures and this conforms to report by Roelf *et al.* (1992) who showed that an optimal temperatures of 30°C and high light are necessary for growth and sporulation of wheat stem rust.

CHAPTER FOUR

EVALUATION OF WHEAT STEM RUST RACES (TTKST, TTKSK, TTTSK AND PTKST) VIRULENCE ON WHEAT STEM RUST DIFFERENTIAL LINES

ABSTRACT

Continued mutation of wheat stem rust makes screening of the previously resistant materials a continuous process. There is continued fear that race Ug99 is still mutating as previously resistant materials are increasingly loosing their resistance. Since most of these resistant materials are currently in breeding programmes, there is an urgent need for constant screening of these materials against any new race reported. A study was therefore conducted at Chepkoilel University College Eldoret, Kenya, to screen wheat differential lines against the races identified in Uasin Gishu region (TTKSK, TTKST, TTTSK and PTKST) during the May-Nov 2009 cropping season. Nine previously resistant differentials against race Ug99 (TTKSK) i.e. *Sr 13, Sr 22, Sr 25, Sr 26, Sr 27, Sr 28, Sr 32, Sr 33* and *Sr 35)* were screened against all the four races identified in Uasin Gishu region. Four *Sr* differentials *(Sr 21, Sr 24, Sr 31* and *Sr, 36)* were also included in the experiment as checks and as confirmatory of the above four races. The trial was conducted in a greenhouse arranged in CRD replicated three times. The scoring was based on the infection types on the leaves after inoculation. The results revealed that race TTKST, which is the predominant race in Uasin Gishu region, was virulent to five of the nine test differentials but *Sr 22, Sr 27, Sr 32* and *Sr 35* appeared to show resistance to this race. Race PTKST was virulent to two of the nine test differentials i.e. *Sr 26* and *Sr 35*. Both race TTKSK and TTTSK were not virulent to any of the nine test differentials. It was therefore concluded that the danger posed by race TTKST and PTKST, which are both virulent to *Sr 24,* gene is real and breeding should emphasize on *Sr* genes that are resistant to these races. The depletion of the sources of resistance by these races calls for more intensified search for other sources of resistance. Moreover with detection of four distinct races, future seedling screening should emphasize on individual race screening.

4.1 Introduction

Historically, stem rust, caused by *Puccinia graminis* f. sp. *tritici*, was one of the most destructive diseases of wheat (*Triticum aestivum* and *T. turgidum* subsp. *durum*) and barley (*Hordeum vulgare*). In recent years, the disease has been under effective control through the widespread use of host resistance. A number of stem rust resistance genes, designated as *Sr* genes in wheat and its close relatives have been described and cataloged (McIntosh, *et al.,* 1995), and monogenic lines carrying individual *Sr* genes are available in several wheat backgrounds. Most of the *Sr* genes have been characterized for their reactions to specific races of *P. graminis* f. sp. *tritici* (Roelf *et al.,* 1992; Jin *et al.,* 2007). Jin *et al.* (2007) reported some monogenic lines which were found to be resistant against race Ug99, unfortunately new mutants of this race have emerged since then as reported by Jin *et al.* (2008) and (2009). Lately the danger posed by these mutants cannot be ignored (Pretoruis *et al.,* 2010). Race TTKST which has been reported to be virulent to *Sr 24* is a mutant of race Ug99 (Jin *et al.,* 2008) and has currently been reported in South Africa (Pretoruis *et al.,* 2010). The danger posed by this new mutant is not well understood. Race TTTKS was reported at Njoro Kenya in 2009 (Jin *et al.,* 2009) and distribution of this race in Kenya is currently unknown, more so its reaction on the previously resistant monogenic lines has not been reported. This study was, therefore, undertaken to determine the virulence of the four races identified in Uasin Gishu region during the May-Nov 2009 cropping season, on previously resistant wheat stem rust differentials.

4.2 Literature review

Race Ug99 has overcome many known *Sr* genes. Genes *Sr5*, *Sr6*, *Sr7a*, *Sr7b*, *Sr8a*, *Sr8b*, *Sr9a*, *Sr9b*, *Sr9d*, *Sr9g*, *Sr10*, *Sr11*, *Sr12*, *Sr15*, *Sr16*, *Sr17*, *Sr18*, *Sr19*, *Sr20*, *Sr23*, *Sr30*, *Sr31*, *Sr34*, *Sr38* and *SrWld-1* (Jin *et al.,* 2007) have all been defeated by race Ug99 (Pretoruis *et al.,* 2000; Jin and Singh, 2006). The additional new virulence has separated isolates of race TTKS into two different races: TTKST virulent to *Sr 31+Sr 24* and TTKST virulent to *Sr 31+Sr 36* (Jin *et al.,* 2008; 2009). This makes the remaining resistant *Sr* genes very vulnerable. Several other genes, including *Sr 13*, *Sr22*, *Sr25*, *Sr 26, Sr 27, Sr 28, Sr 32, Sr 33, Sr35*, *Sr39*, *Sr40* and *Sr44* identified from different wheat relatives, are moderately to highly resistant to race Ug99 at

seedling stage (Jin *et al.,* 2007; Singh *et al.,* 2007). These genes are currently being transferred into wheat backgrounds to develop commercial cultivars. They are good sources of resistance genes for control of race Ug99, but their reaction against the mutants of race Ug 99 is currently unknown.

4.3 Materials and Methods

4.3.1 Study site

The study was carried out in a greenhouse at Chepkoilel University College which is located at 0.57N; 35.31E and at altitude of 2143.6 m.a.s.l.

4.3.2 Evaluation of stem rust races virulence on differential lines

The races identified in Uasin Gishu region (TTKSK, TTKST, TTTSK and PTKST) were screened on nine race Ug99 resistant wheat differentials (*Sr 13, Sr 22, Sr 25, Sr 26, Sr 27, Sr 28, Sr 32, Sr 33* and *Sr 35)* (Jin *et al.,* 2007). Four differentials (*Sr 21, Sr 24, Sr 31* and *Sr 36*) were also included as checks to confirm the above four races.

Race symptomatology and Infection Type was scored on the 0-4 scale as proposed by Stakman *et al.* (1962). The thirteen stem rust wheat differentials were each planted in plastic pots filled with a mixture of sterile silt clay, sand and forest soil. Each pot was planted with three seeds of the same line and the trial was arranged in a complete randomized design (CRD) replicated three times. Each line was screened against the four races; inoculation, incubation and scoring was as described in chapter three.

4.4 Results and Discussion

The results of this study as shown in Table 8 revealed that, some of the previously resistant differentials (Jin *et al.,* 2007) are currently susceptible to mutants of race Ug99. *Sr 13* which was previously resistant produced susceptible infection type to race TTKST, however the gene was resistant to original race Ug99 (TTKSK), TTTSK and PTKST. *Sr 22* produced resistant infection types when tested against all the four races. *Sr 25* produced susceptible infection types on race TTKST but the differential was resistant against TTKSK, TTTSK and PTKST.

Table 8: Infection types (ITs) on seedling of differential stem rust resistance gene lines against race TTKSK, TTKST, TTTSK and PTKST of *Puccinia graminis* f. sp. *tritici*

Differential	Sr gene	Seedling ITs			
Check		**TTKSK**	**TTKST**	**TTTSK**	**PTKST**
CS_T_mono_deriv	21	3	4	3-	1
LcSr24Ag	24	2	3+	;	3+
Sr31 (Benno)/6*LMPG	31	4	4	3+	4
W2691SrTt-1	36	;	0	3+	2-
Test					
Combination VII	13	2-	3	2-	2
Kingbird	13	2+	3	2-	2
T. mono.Deriv./9*LMPG	22	1	2-	2	1
LcSr25Ars	25	2	3+	1	2
PW327/4*Tc//9*LMPG	26	1	3-	;	;
Eagle (Australia)	26	1	3-	;	1
73,214,3-1/9*LMPG	27	;	2+	;	;
W2691/Sr28Kt	28	;	3+	;	;
CnsSr32 A.s.	32	1	1	1	2
RL 5405	33	2	3+	2	;
Mq(2)5*G2919	35	;	2-	;	3

0, ;, 1 and 2 =Resistant; 3 and 4 =Susceptible; + large pustules; - small pustules

Sr 26 produced susceptible infection type to race TTKST, but resistant infection types to race TTKSK, TTTKS and PTKST. *Sr 27* was resistant to all the four races. *Sr 28* produced a susceptible infection type when race TTKST was used, and was resistant to the other three races. *Sr 32* produced resistant infection type to all the four races used. *Sr 33* produced a susceptible infection type when race TTKST was used, and was resistant to the other three races. *Sr 35* produced susceptible pustules to race PTKST and was resistant to the other three.

The study as shown in Table 8 revealed that race Ug99 (TTKSK) and its mutant race TTTSK *(Ug99+Sr36)* are still not virulent to the nine test differentials, whereas two mutants; race TTKST *(Ug99+Sr 24)* and race PTKST *(Ug99+Sr24-Sr21)* were virulent to some of the previously resistant wheat differentials. Race TTKST *(Ug99+Sr 24)* was virulent to seven of the eleven test differentials used in this study, while race PTKST *(Ug99+Sr24-Sr21)* was virulent to one test differential *(Sr 35).* The study showed how mutants of race Ug99 pose a threat to the previously resistant materials. Races which are virulent to *Sr24* are rare in the world but lately it has been reported in South Africa (Pretoruis *et al.,* 2010). The significance of new mutants of race Ug99 is therefore a real threat to wheat breeding. Since new mutants of race *Ug99* are being reported currently (e.g. PTKST) it is important for any resistant material to be tested against these races, more so the depletion of the source of resistance calls for research on identifying other sources of resistance.

CHAPTER FIVE

EFFECT OF RATES AND TIME OF APPLICATION OF TEBUCONAZOLE 250 g/L + TRIDIMENOL 125 g/L (*Silvacur 375g/L)* FUNGICIDE IN CONTROL OF WHEAT STEM RUST *(Puccinia graminis* f.sp *tritici)* AND YIELDS

ABSTRACT

Stem rust is most severe 60 days after planting onwards up to grain hardening and the most sensitive period is from booting up to grain filling. As part of the integrated disease management, chemical control continues to play an important role in control of stem rust. An experiment was therefore carried out to evaluate the effect of rates and time of fungicide (Silvacur 375g/L) application based on initial visual disease severity under a single spray regime. The trial was carried out for two seasons i.e. short rain of 2009 and long rain of 2010 at KARI Njoro. The trial was arranged in a split plot design, where the rates of 0 L/ha (R_0), 0.5 L/ha ($R_{0.5}$), 0.75 L/ha ($R_{0.75}$) and 1 L/ha(R_1) were the main plots and the time of fungicide application based on initial stem rust severity at 5% (T_5), 10% (T_{10}) and 20% (T_{20}) were the sub plots. During the short rain season (low disease pressure), $R_{0.75}T_{10}$, R_1T_5 and R_1T_{10} gave a grain yield of 3.05 t/ha, 3.18 t/ha and 3.28 t/ha respectively, these were found to be significantly equal to the positive control R_{1C} which had been sprayed thrice at booting, heading and flowering giving a grain yield of 3.83 t/ha, against the untreated plot R_0 which gave 1.01 t/ha. During the long rain season (high disease pressure) R_1T_{10} had a grain yield of 3.84 t/ha which was significantly equal to the positive control R_{1C} which had a grain yield of 4.3 t/ha against the untreated plot that had 0.66 t/ha. The correlation data also revealed that control of the disease at different growth stages of the crop had a significant effect on both grain yield and test weight. The study therefore found out that both rates and time of application of fungicide is very important for a single spray regime to be effective. It was therefore recommended that it is possible to control wheat stem rust using a single spray regime when a rate of 0.75-1 L/ha and a timing of 5-10% initial disease severity is adopted.

5.1 Introduction

As part of the integrated disease management, chemical control continues to play an important role in control of stem rust. Since the release of a variety takes many years and the possibility of genes breakdown (Park, 2008), there is need to have effective fungicides, rates and timing of application thus developing a consolidated spray regime. The use of fungicides has been reported to effectively control stem rust in wheat (Wanyera *et al.,* 2009). Stem rust is most severe 60 days after planting onwards up to grain hardening and the most sensitive period is from booting up to grain filling. Considering the cost involved in each spray, it is important to study the losses associated with different application rates, timing and also the frequency of spray during this period. In comparison with other wheat fungicides Silvacur 375 EC is currently recommended for control of wheat stem rust (Wanyera *et al.,* 2009). However, the high cost of chemicals application (Table 9) and environmental concerns, require research to concentrate on minimum number of application frequencies, low rates and effective timing. For these reasons a study was conducted to determine the effect of Silvacur 375 EC rates and time of application under a single spray regime on wheat stem rust and yields.

5.2 Literature review

Several stem rust control measures have been adopted both hear in Kenya and elsewhere (Table 9). It is worth noting that no single stem rust control method is effective without the support of the others and therefore farmers should aim at a holistic approach of combining resistance, cultural and fungicides to maximize their benefits.

Diseases of agricultural crops continue to cause severe losses and to pose a threat to food security and to the sustainability of crop production across large regions of the world (Strange & Scott, 2005; Oerke, 2006; Gilligan, 2008). Some of these threats involve emerging or re-emerging pathogens, exemplified by new strains of wheat stem rust (Wanyera *et al.,* 2006) in Africa, Asia and the Middle East. The risks, however, of severe enough outbreaks to merit control over large regions often vary from year to year. Such variability reflects the inherent stochasticity of epidemics as well as uncertainties associated with weather patterns. For many

crop diseases, chemical control remains the principal means of disease reduction and eradication (Cook *et al.,* 1999; Paveley *et al.,* 2008). Chemical control encompasses a wide range of pesticides and fungicides. Whereas routine application of chemical control may once have been possible when labour and materials were comparatively cheap, increasing costs together with concerns about hazards to non-target organisms and the risks of selecting for fungicide resistance in target populations militate against routine use in many crops (Waard *et al.,* 1993). How and when, if at all, to deploy expensive methods for disease control over large regions in the face of uncertainty remains a major scientific challenge. The challenge lies in combining epidemiological models for disease outbreaks with economic models in the presence of uncertainty, in order to identify the optimal timing to apply control. Clearly, failure to control early enough when an outbreak occurs can lead to severe crop loss. Unnecessary deployment also incurs direct losses through costs of fungicides, pesticides and additional labour; it may also lead to indirect costs when over-use of chemical results in selecting for resistant strains in the target population.

Table 9: Methods of controlling the rust diseases (Roelf *et al.*, 1992)

Control method	Controlled by	Cost to	Cost	Effectiveness
Gene pyramids	Breeder	Taxpayer/Seed buyer	Low	Good
Gene deployment	Breeder group	Taxpayer/Seed buyer	Moderate	Good
Multilines	Breeder	Taxpayer/Seed buyer	High	Fair to good
Cultivar mixtures	Seed producer	Seed buyer	Moderate	Fair to good
Chemical	Grower	Grower	High	Good
Cultural	Grower	Grower	Low	Fair
Eradication of alternate host	Legal system	Taxpayer	High	Fair to good

Chemical control has been successfully used to control rust disease in wheat in China (Wang *et al.,* 2008) and Europe permitting high yields of 6-7 t/ha (Buchenauer, 1982; Simmonds and Rajaram, 1988). In Southern United State chemicals have been only permitted when yields exceeding 2 t/ha is expected while in Brazil and Paraguay yields of more than 1 t/ha justifies the use of chemical. In Kenya such studies are needed so that the farmers can have economical spray regimes. Stem rust is most severe from booting up to grain filling and this makes its spray programme unique as most pests and other rust diseases at this time are not severe. However, this is the period that has been reported to be the best in terms of foliar fungicide uptake and fungicides applied prior to flag leaf emergence have been shown not to reduce t_o (initial disease infection) and r (rate of infection) (Paveley, 2000). The interception of foliar-applied fungicide depends, amongst other factors, on the planar area and orientation of leaves at the time of spray application (Grayson & McCarthy, 1987). Prior to emergence, direct interception will be negligible. During the early stages of emergence, leaves are vertical and rolled, presenting a small area to spray droplets. As systemic movement of ergosterol biosynthesis-inhibiting fungicides is predominantly acropetal within the leaf to which they are applied, fungicide applications before a leaf is fully emerged allow subsequent infections on that leaf to escape much of the dose applied (Buchenauer, 1987; Pommer, 1987). Furthermore, the concentration of fungicide or active metabolites declines over time, so the dose encountered by the pathogen depends on both the dose applied and the period between application and infection.

The effect of rates and time of spray has been studied in other crop as reported by Yorinori (2004), where it has shown great effect on the disease progress and ultimately affecting the yields. Optimizing the timing of a single application of a fungicide has shown to be of economic importance to the farmer considering the cost involved with spraying (Martial *et al.,* 2010) more so if there are some resistant varieties (Groth, 2008).

5.3 Materials and Methods

5.3.1 Trial layout

A local commercial grown wheat *var Duma* was planted at KARI-Njoro in Nov 2009 (short rain season) and May 2010 (long rain season) in a Split plot RCBD with three replications. A precision planter was used where seed rate of 125kg/ha in 8 rows 20cm apart and Di-ammonium

phosphate (N: 18% P: 46% K: 0%) fertilizer was applied at a rate of 150kg/ha. Each plot was 1.5 wide by 3m long. A spreader row was planted all round the plot and later inoculated by dusting them with urediospores of *Puccinia graminis* f. sp. *tritici* mixed with talc powder at the beginning of stem elongation, (GS 31) as outlined by McIntosh *et al.* (1995).

The treatment fungicide, Tebuconazole 250g/L+ Tridimenol 125g/L *(Silvacur 375 EC)* at four rates i.e. 0 L/ha (R_0), 0.5 L/ha ($R_{0.5}$), 0.75 L/ha ($R_{0.75}$), 1.0 L/ha (R_1), was used as the main plot whereas the time of applications i.e. at 5% infection (T_5), 10% infection (T_{10}), and 20% infection (T_{20}) was the sub plots. The positive control treatment (Rc) was sprayed as described by Wanyera *et al.* (2009) i.e. a rate of 1 L/ha at GS 45 (booting), GS 55 (heading), and GS 65 (flowering) to ensure no disease at all during the crop growth. Untreated plot (R_0) acted as a negative control.

Normal wheat agronomic practices were followed as described by Kinyua and Ochieng (2005), where weeds were controlled at the end of tillering using Buctril MC (Bromoxynil Octanoate 225g/L and MCPA Ethyl Hexyl Ester 225g/L) at 1.25 L/ha mixed with Puma komplete (Fenoxaprop-P-ethyl 64g/L+ Iodosulfuron-methyl-sodium 8g/L+ Mefenpyr-diethyl 24g/L) for both grass and broad leaf weeds. Aphids were controlled using Bulldock star EC (Beta-Cyfluthrin 12.5g/L+ Chlorpyrifos 250g/L) at end of tillering and another at stem elongation which was also tank-mixed with a foliar fertilizer Byfollan (11%N, 8%P_2O_5, 6%K2O) at a rate of 20ml/ha.

5.3.2 Treatment application

Ten plants were randomly selected in each plot (Tedesse *et al.,* 2010) and when 5% of the disease severity was observed based on the modified cobs scale of 0-100%, all the rates were applied on the T_5 plots only. As the disease progressed to 10% on the remaining plots all the rates were applied on the T_{10} plots only and then as the disease reached 20% on the remaining plots all the rates were applied on the T_{20} plots. During treatment application, boards were used to prevent drift to other plots.

5.3.3 Data Collection and analysis

5.3.3.1 Rust Severity Assessment

5.3.3.1.1 Weekly stem rust severity: Severity was recorded using the modified Cobb's scale (Peterson *et al.*, 1972), by assessing 10 randomly pre-tagged plants. The average stem rust severity from the 10 plants of each plot was used for analysis (Tedesse *et al.*, 2010). During disease assessment, the growth stage of the crop was recorded to observe onset and progress of the disease in relation to wheat phenology. From this, disease severity at heading (GS 55), flowering (GS 65) and milk stage (GS 77) was developed, which was used for analysis. Crop growth stage was assessed based on the decimalized key developed by Zadoks *et al.* (1974).

5.3.3.1.2 Final Disease Score (FDS): Was considered to be the final stage during which the wheat crop is susceptible to wheat stem rust usually at GS 85 (soft dough stage).

5.3.3.2 Epidemic evaluation and analysis

5.3.3.2.1 Area Under Disease Progress Curve (AUDPC): This was calculated using a computer programme developed at CIMMYT following the formula given by Roelfs *et al.* (1992), which calculates the area of the curve created by the disease scores taken at GS 55 (heading), GS 65 (flowering) and GS 77 (late milk) (Lal Ahamed *et al.*, 2004).

5.3.3.3 Yield Measurements

5.3.3.3.1 Test weight (1000-kernel weight): This was determined after counting the grains with an electronic seed counter (contador pfeuffer®). The 1000 seeds obtained were then weighed using a digital weighing balance.

5.3.3.3.2 Grain yield: Harvesting was done using the Hans-Ulrich Hege plot combine harvester (Saatzuchtmaschinen, Hohebuch) and the harvest was converted to yields/ha.

5.3.3.4 Data analysis

The data was subjected to statistical analysis using MSTATC statistical package and means separated using least significant difference.

5.3.4 Results and Discussion

5.3.4.1 Effect of Silvacur 375 g/L rates and time of application on test weight and grain yield of wheat *cv Duma*

The results summarized in Table 10 revealed that; the positive control R_{1c} where Silvacur was applied at a rate of 1 L/ha three times at growth stages (GS) 45, 55 and 65, gave the highest ($P \leq 0.05$) grain yield in both seasons. The grain yield recorded was 3.832 and 4.298 t/ha which was 73.8 and 84.6% increase against the untreated for season one and two respectively. R_1 gave a grain yield of 3.037 and 3.548 t/ha which was 66.7% and 81.4% increase against the untreated for season one and two respectively. $R_{0.75}$ gave 2.625 and 2.870 t/ha which was 61.7% and 77% increase against the untreated for season one and two respectively, $R_{0.5}$ gave 1.874 and 1.848 t/ha which was 46.3 and 64.2% increase against the untreated for season one and two respectively whereas the untreated plot (R_0) gave 1.006 and 0.661 t/ha for season one and two respectively. Similar trends were observed on the test weight, where the positive control (R_{1c}) gave the best test weight. A correlation analysis (Table 16) showed that there was a highly significant ($P \leq 0.05$) positive correlation ($r=0.724$) of grain yield and test weight. This implies that it is possible to predict the grain yield based on the test weight data and vice versa. A comparison of grain yield and test weight for both season revealed a general increase for season two data on the controlled plots, which could be attributed to the high rainfall coupled with disease suppression using the fungicide. However, the same (second season) reported a decline in grain yield on the untreated (R_0), attributed to high disease pressure during this season (Table 11) which resulted from high rainfall (Appendix 5), thus high relative humidity, which is the conducive environment for rust development (Roelf *et al.*, 1992).

The effect of time of applying Silvacur to wheat as given in Table 10, shows that a single application of Silvacur when the visual disease severity was at approximately 10% (T_{10}) on crop canopy resulted in higher grain yield of 2.757 t/ha and 2.959 t/ha which was 9.4 and 13.2% increase than when the treatment was applied at T_5 and 21.4 and 18.7% more than when the treatment was applied at T_{20} for season one and two respectively. Therefore application at T_{10} gave a grain yield which was significantly higher ($P \leq 0.05$) than T_5 which gave 2.499 and 2.569

t/ha or T_{20} which gave 2.168 and 2.645 t/ha for season one and two respectively. However the effect of time of applying Silvacur to wheat on test weight revealed that late single application of Silvacur (20% disease severity) resulted in higher test weight of 31.81 and 31.69 g in season one and two respectively which was significantly ($P \leq 0.05$) higher than when a single spray was done at 5% (30.18 and 30.17g) or 10% (31.28 and 30.27g) disease severity. This was found to be 5.12-4.78% more than spraying at T_{10} and 4.48-4.95% more than spraying at T_5.

Table 10: Effect of Silvacur 375 g/L rates and time of application on test weight (g) and grain yield (t/ha)

	Test weight (g)		Grain yield (t/ha)	
	Season		Season	
Treatment	**1**	**2**	**1**	**2**
Rates				
R_0	27.17*d*	24.80*e*	1.006*d*	0.661*e*
$R_{0.5}$	29.48*c*	28.64*d*	1.874*c*	1.848*d*
$R_{0.75}$	30.63*c*	30.30*c*	2.625*b*	2.870*c*
R_1	33.33*b*	32.77*b*	3.037*b*	3.548*b*
R_{1C}	34.82*a*	37.04*a*	3.832*a*	4.298*a*
Means	31.09	30.71	2.475	2.645
C.V	0.8	0.6	6.4	3.1
LSD *($P \leq 0.05$)*	1.195	2.125	0.595	0.3627
Time				
T_5	30.18*b*	30.17*b*	2.499*ab*	2.569*b*
T_{10}	31.28*ab*	30.27*b*	2.757*a*	2.959*a*
T_{20}	31.81*a*	31.69*a*	2.168*b*	2.407*c*
Means	31.09	30.71	2.475	2.645
C.V	2.0	3.7	12.8	7.3
LSD *($P \leq 0.05$)*	1.417	0.836	0.448	0.1555

Treatment means within columns followed by the same letter are not significantly different at *$P \leq 0.05$* LSD test

The test weight data revealed that, fungicide efficacy during the milk stage all the way to soft dough is very critical for quality seeds to form. Therefore under a single spray regime early spray should be avoided as this will lead to re-infection during the later stages and thus low quality grains with very low test weight. The spray timing of 20% initial disease severity, which coincided with milk stage, ensures the existence/presence of Silvacur through the critical period of grain filling stage to maturity.

5.3.4.2 Effect of Silvacur 375 g/L rates and time of application on final disease severity (FDS) recorded at soft dough stage (GS 85) and area under disease progress curve (AUDPC)

The results summarized in Table 11 revealed that, the positive control R_{1c} where Silvacur was applied at a rate of 1 L/ha three times i.e. GS 45, GS 55 and GS 65, gave the lowest ($P \leq 0.05$) FDS at soft dough stage (GS 85) of wheat in both seasons. Final disease severity (FDS) recorded was only 3 and 6% for season one and two respectively, compared to 23 and 27% for R1, 37 and 38% for R0.75, 43 for R0.5, 76 and 79% for R0 for season one and two respectively. The degree of stem rust severity correlated highly ($r=0.869$) at GS 77 and GS 85 ($r=0.957$) (Table 16) with the AUDPC, where treatments with higher disease severity recorded higher AUDPC (Table 11) in both seasons. Comparison of disease severity and AUDPC in both season were lower for season one and this could be attributed to low rainfall regimes experienced during the first season (Appendix 5), thus low relative humidity which is crucial for wheat stem rust development (Roelf *et al.*, 1992).

The effect of time and rates of applying Silvacur on wheat stem rust severity and AUDPC is given in Table 11. The data shows that a single spray of Silvacur applied when the visual disease severity was at approximately 10% on crop canopy, resulted in lower disease pressure of 30 and 35% in season one and two respectively, which was significantly lower ($P \leq 0.05$) than spraying earlier (5% visual disease severity) which gave 37% and 39% or spraying later (20%) which gave 43 and 41 for season one and two respectively.

Table 11: Effect of Silvacur 375 g/L rates and time of application on FDS i.e. GS 85 or soft dough stage and AUDPC on wheat crop at KARI-Njoro during 2009 and 2010 cropping seasons

	Final disease severity (FDS)		Area Under Disease Progress Curve (AUDPC)	
	Season		Season	
Treatment	**1**	**2**	**1**	**2**
Rates (L/ha)				
R_0	75.6*d*	78.9*e*	1394*c*	1878*e*
$R_{0.5}$	43.3*c*	43.3*d*	1021*bc*	1090*d*
$R_{0.75}$	36.7*c*	37.8*c*	939*bc*	870*c*
R_1	23.3*b*	26.7*b*	627*ab*	657*b*
R_{1C}	3.2*a*	6.1*a*	97*a*	77*a*
Means	36.4	38.6	815	914
C.V	7.2	3.9	16.3	21.7
LSD*(P≤0.05)*	8.010	4.131	700.3	55.6
Time				
T_5	36.5*b*	39.3*b*	741*a*	957*b*
T_{10}	30.1*a*	35.3*a*	700*a*	794*a*
T20	42.7*c*	41.0*c*	1005*a*	992*b*
Means	36.4	38.6	815	914
C.V	11.7	5.7	25.6	23.2
LSD*(P≤0.05)*	3.57	3.61	704.3	54

Treatment means within columns followed by the same letter are not significantly different at *P≤0.05* LSD test

5.3.4.3 Interaction effect of rates and time of application of Silvacur 375 g/L on test weight and grain yield

The results as given in Table 12a and 12b shows that control treatment R_{1c} and R_0 gave the highest (3.83 and 4.3 t/ha) and the lowest (1.01 and 0.66 t/ha) grain yield for the seasons one and two respectively. However, a single spray of Silvacur (1 L/ha) given at T_5 and T_{10} produced grain yields of 3.18 and 3.28 t/ha which was significantly ($P \leq 0.05$) equal to the positive control. During the second season only R_1T_{10} gave a grain yield (3.84 t/ha) significantly ($P \leq 0.05$) equal to the positive control. A rate of 0.75 L/ha applied when the initial disease severity was at 10%, ($R_{0.75}T_{10}$) gave a grain yield of 3.05 t/ha which was also significantly similar to the positive control. In the second season however, this rate did not give a grain yield equal to the positive control (R_{1c}).

Table 12a: Interaction effects of rates (L/ha) and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on Grain yields (t/ha) at KARI-Njoro 2009 cropping season.

	R0.5	R0.75	R1	R1c	R0
T5	2.03*de*	2.5*bcd*	3.18*abc*	3.83*a*	1.01*f*
T10	2.41*bcd*	3.05*abc*	3.28*ab*		
T20	1.18*ef*	2.23*cd*	2.66*bcd*		
C.V	23.8				
L.S.D	0.9616				

Table 12b: Interaction effects of rates (L/ha) and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on Grain yields (t/ha) at KARI-Njoro 2010 cropping season.

	R0.5	R0.75	R1	R1c	R0
T5	1.61*e*	2.93*c*	3.44*b*	4.3*a*	0.66*f*
T10	2.31*d*	3.45*b*	3.84*a*		
T20	1.62*e*	2.23*d*	2.82*bc*		
C.V	7.7				
L.S.D	0.4339				

Treatment means followed by the same letter are not significantly different at LSD $P \leq 0.05$

Although the positive control (R_{1C}) gave the highest grain yield and test weight, significantly equal yields were observed under certain single spray regime. The study also revealed that during the short rain season it is possible to have a low rate of 0.75 L/ha applied at 10% disease severity, while during the long rain season only the high rate of 1 L/ha applied at either 5 or 10% disease severity gave grain yield equal to that of positive control.

The best interaction of a single spray regime for control of stem rust was a rate of 0.75 L/ha and 1 L/ha depending on season. This is higher than the manufactures' earlier recommendation of 0.5 L/ha, where the product was recommended for the control of other fungal diseases and not stem rust. Moreover, the virulent races of wheat stem rust used in this study had not been reported. The study of effect of spray timings revealed that the best time of application under a single spray regime was when the disease was at 10% (Table 11) which ultimately resulted in high yields (Table 10 and 12a and 12b). This coincided with the heading stage all the way up to soft dough stage. This finding concurs with studies by McGrath and Pennypacker, (1991) who showed that carbon dioxide fixation after flowering supplies 90-95% of the carbohydrate in the grain. These carbohydrates are translocated from the health flag leaf to growing reproductive organs and ultimately grains. Therefore, the high grain yield reported when a rate of 0.75 L/ha -1 L/ha is applied when the initial disease severity is between 5-10% shows that control of the

disease at this critical period of booting (GS 65) will have a better impact on the test weight and grain yields.

The results on test weight as shown in Figure 13a and 13b reveals similar trends as for grain yield where the best test weight was observed under the positive control which had 35.37 and 37.39 g/1000 kernel, for season one and two respectively. This was significantly ($P \leq 0.05$) equal to R_1T_{10} and R_1T_{20} which had 34.33 and 35.60 g/1000 kernel respectively during the first season. During the second season only R_1T_{20} (35.23 g), gave a test weight significantly ($P \leq 0.05$) equal to the positive control. The data shows that there is a general increase in test weight when the rates are increased from 0.5 L/ha to 0.75 L/ha. Furthermore, an increase in test weight was observed when the single spray was delayed to be given at 20% initial disease severity than when applied early at 5% disease severity. Therefore, lower test weights (23.97g) were observed when lower rates of Silvacur were applied at lower initial disease severities, which was significantly equal to the negative control that was not sprayed at all (24.94g). The test weight of wheat grain ranged between 32.8-34.8g for the 1 L/ha of Silvacur sprays and this correlated highly ($r=0.724$) with grain yields. Macharia, (2009) reported a significant high correlation ($r=0.75$) of grain yield and final disease severity thus confirming that these two parameters are related. The data also points out these critical periods which affect the yield. It was apparently clear that early disease infection i.e. at GS 55 (heading) did have a significant and negative correlation to the yield. However, as the disease progressed to GS 65 (flowering) there was a highly significant ($P \leq 0.01$) negative correlation on grain yield, but only significant ($P \leq 0.05$) negative correlation to 1000 kernel weight.

Table 13a: Interaction effects of rates (L/ha) and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on 1000 kernel weight at KARI-Njoro 2009 cropping season.

	R0.5	R0.75	R1	R1c	R0
T5	23.97*g*	27.77*de*	26.97*ef*	35.37*a*	24.94*fg*
T10	31.20*bc*	30.89*bc*	34.33*a*		
T20	29.40*cd*	31.93*b*	35.60*a*		
C.V	5.0				
L.S.D	2.334				

Table 13b: Interaction effects of rates (L/ha) and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on 1000 kernel weight at KARI-Njoro 2010 cropping season.

	R0.5	R0.75	R1	R1c	R0
T5	23.33*h*	26.37*fg*	27.00*ef*	37.39*a*	24.80*gh*
T10	28.57*def*	31.23*c*	34.33*b*		
T20	29.00*cde*	29.97*cd*	35.23*ab*		
C.V	3.5				
L.S.D	2.502				

Treatment means followed by the same letter are not significantly different at LSD $P \leq 0.05$

Finally as the disease approached GS 77 (milk stage) apart from the grain yield, the 1000 kernel weight was also highly significant ($P \leq 0.01$), indicating how this stage is important in kernel weight. This is further supported by the report of Bartlett *et al.* (2002) who showed that the green area duration between heading to maturity is closely correlated to wheat grain yield.

5.3.4.4 Interaction of Silvacur 375 g/L application rates and timing on final disease severity (FDS) and area under disease progress curve (AUDPC) of wheat grown at KARI, Njoro

The results as summarized in Table 14a and 14b shows that control treatment R_{1c} and R_0 gave the best (3 and 6%) and the worst (76 and 79%) stem rust severity infection levels at soft dough

stage of wheat for the two seasons respectively. Single sprays of Silvacur at the rate of 1 L/ha when the initial visual disease severity was 10% (R_1T_{10}), maintained the disease progress to 20% by FDS (GS 85), which was the lowest threshold in both seasons. For season I however, similar results were obtained when Silvacur at 1 L/ha was also sprayed initially when visual disease infection was 5% (R_1T_5). Similar level of control was obtained when a single spray of Silvacur at the rate of 0.75 L/ha was applied when initial disease severity was at 10% ($R_{0.75}T_{10}$) during the first season. However, during the second season the same rate of 0.75 L/ha at 10% initial disease severity gave a significantly higher final disease severity of 37%. When Silvacur was applied at a rate of 0.5 L/ha the best timing was at T_{10}, then T_5 and T_{20} which had a severity of 33, 43 and 53% respectively, however during the second season, $R_{0.5}$ at T_5, T_{10} and T_{20} recorded no significant ($P \leq 0.05$) effect on the final disease severity .

A comparison of the two seasons shows a general increase on the FDS during the second season which was mainly attributed to high relative humidity due to higher rainfall observed during this particular season. The results of FDS correlated positively and significantly ($r=0.957$) to the AUDPC (Table 16) where the treatments with significantly ($P \leq 0.05$) low FDS (R_{1C}) gave the least AUDPC (96 and 77), whereas the treatments with significantly ($P \leq 0.05$) highest FDS (R_0), gave the highest AUDPC of 1393 and 1878 for the two seasons respectively. A comparison of the treatments interaction revealed that a rate of 1 L/ha applied at 5% and 10% initial disease

Table 14a: Interaction effect of rates and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on Final Disease Severity (FDS) at KARI-Njoro 2009 season.

	R0.5	**R0.75**	**R1**	**R1c**	**R0**
T5	43.3*fe*	40.0*ed*	20.0*b*	3.2*a*	75.56*h*
T10	33.3*dc*	20.0*b*	20.0*b*		
T20	53.3*g*	50.0*gf*	30.0*c*		
C.V	12.9				
L.S.D	9.725				

Table 14b: Interaction effect of rates and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on Final Disease Severity (FDS) at KARI-Njoro 2010 season.

	R0.5	R0.75	R1	R1c	R0
T5	46.7*e*	33.3*c*	30.0*c*	6.1*a*	78.89*f*
T10	33.3*e*	36.7*dc*	20.0*b*		
T20	50.0*e*	43.3*ed*	30.0*c*		
C.V	12.3				
L.S.D	7.448				

Treatment means followed by the same letter are not significantly different at P≤0.05 LSD test.

severity gave AUDPC that was significantly (P≤0.05) equal to the positive control i.e. 452 and 482 respectively during the first season. However, during the second season the same rate at 5% and 10% initial disease severity gave AUDPC that was significantly different from that of positive control i.e. 672 and 442, respectively.

Final disease severity at GS 85 (soft dough) of Duma wheat variety, had a high correlation coefficient (r=0.869) with AUDPC, test weight (r=0.782) and grain yield (r=0.760) (Table 16). For these reasons the Silvacur treatment application regimes (rate and time) that reduced stem rust infection by early milk stage and /or soft dough stages gave significantly higher test weight and grain yield of Duma wheat grown in Njoro. Wheat grain yields of over 3 tones/ha were obtained with Silvacur spray treatment of 1 L/ha (Table 12). These treatments had the lowest disease severity by soft dough stage ranging between 3-27% (Table 14). Wanyera *et al.* (2009) reported a similar trend, where the treatments with lowest disease severity at soft dough results in higher grain yield.

Table 15a: Interaction effects of rates (L/ha) and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on Area Under Disease Progress Curve (AUDPC) at KARI-Njoro 2009 cropping season.

	R0.5	R0.75	R1	R1c	R0
T5	922*dcd*	885*bcd*	452*ab*	96*a*	1393*d*
T10	852*dcd*	684*abc*	482*ab*		
T20	1287*cd*	1250*cd*	948*bcd*		
C.V	10.5				
L.S.D	704.3				

Table 15b: Interaction effects of rates (L/ha) and application time of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) on Area Under Disease Progress Curve (AUDPC) at KARI-Njoro 2010 cropping season.

	R0.5	R0.75	R1	R1c	R0
T5	1270*g*	804*d*	672*c*	77*a*	1878*h*
T10	876*d*	811*d*	442*b*		
T20	1125*f*	995*e*	856*d*		
C.V	7.7				
L.S.D	108.8				

Treatment means followed by the same letter are not significantly different at $P \leq 0.05$ LSD test.

5.3.4.5 Effect of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) rates and time of application on disease progress at different crop growth stages (GS 55, GS 65 and GS 77).

The results summarized in Figure 5 shows that the disease progress was greatly affected by the rates of Tebuconazole 250g/L +Tridimenal 125g/L (Silvacur 375 g/L) used. The positive control plot (R_{1c}) where Silvacur 375 g/L was applied three times gave the best level of control during the entire crop growth i.e. up to Growth Stage (GS) 85 (Dough stage) which was about 109 Days After Planting (DAP). When a single spray of Silvacur 375 g/L was applied at a rate of 1 L/ha, the visual disease severity was maintained (efficacy period) up to GS 75 (milk stage) which was

about 95 DAP, when a rates of 0.75 L/ha was applied the efficacy period lasted up to GS 65 (mid flowering) which was about 80 DAP, when a rate of 0.5 L/ha was applied the efficacy period lasted up to GS 60 (Beginning of flowering) which was about 70 DAP.

Figure 6 shows that the time of Silvacur application based on the visual disease severity affected the disease progress. When Silvacur 375 g/L was applied when the disease severity was 5% (T_5), the visual disease severity was reduced and it took up to 14 days before the re infection reached a visual severity of 5% (efficacy period), this period was between GS 45 (Booting) up to GS 60 (beginning of flowering). When Silvacur 375 g/L was applied at (T_{10}) the efficacy period lasted 11 days before the re infection reached a visual severity of 10%, this period was between GS 55 (Ear emerged) up to GS 65 (Mid flowering). When the treatment was applied at T_{20} the efficacy period lasted 7 days before the re infection reached a visual severity of 20% this period was between GS 65 (Mid flowering) up to GS 75 (Mid milk).

Figure 5: Effect of Rates (L/ha) of Tebuconazole 250g/L+ Tridimenal 125g/L (Silvacur 375 g/L) on Disease severity at different growth stages of wheat crop.

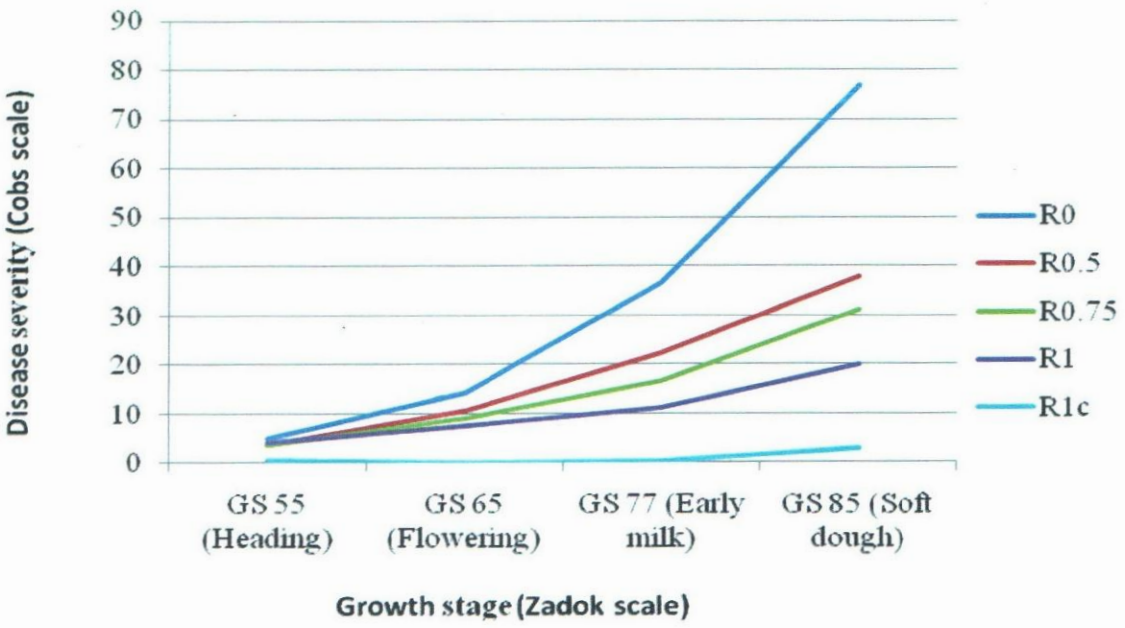
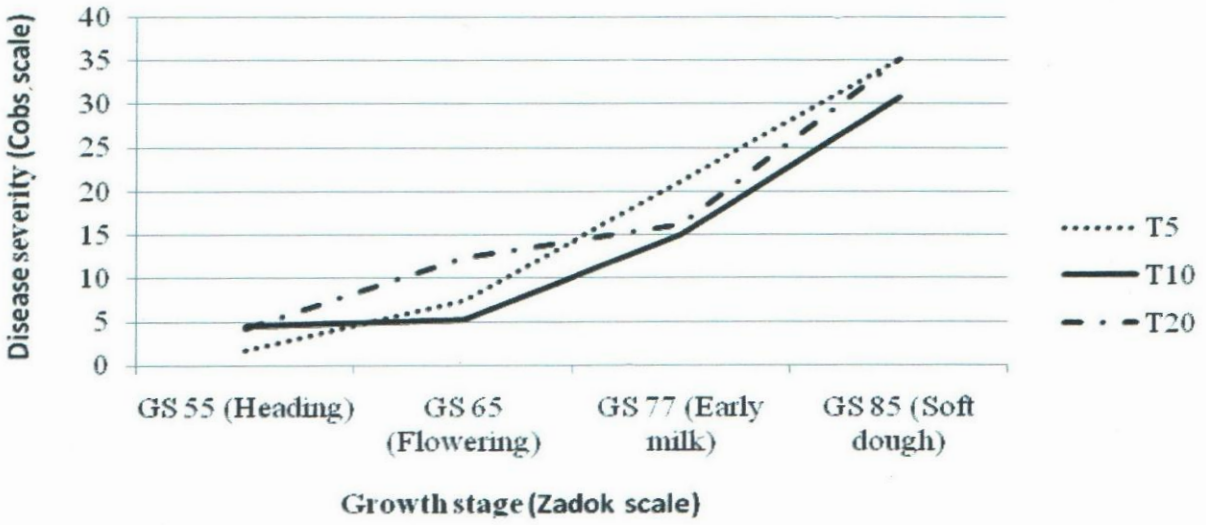


Figure 6: Effect of Time of application of Tebuconazole 250g/L + Tridimenal 125 g/L (Silvacur 375 g/L) based on visual stem rust severity in crop.



5.3.4.6 Correlation coefficient relationship between disease severity, AUDPC and yield parameters of wheat crop.

5.3.4.6.1 Correlation coefficient relationship of disease severity at GS 55 (heading)

Correlation coefficient (r) among wheat stem rust parameters and effect on yield at KARI Njoro during the 2009 and 2010 cropping seasons is given in Table 16. Pearson's correlation coefficient showed that wheat stem rust disease severity at early stage of heading (GS 55) was not significantly ($P \leq 0.05$ and $P \leq 0.01$) correlated to disease severity in later stages, AUDPC and yield parameters of wheat crop. This implied that under a single spray regime of Silvacur 375 g/L, symptoms of the disease at this stage will not cause any significant effect to subsequent disease severity and the yields parameters.

5.3.4.6.2 Correlation coefficient relationship of disease severity at GS 65 (flowering)

Disease severity at flowering (GS 65) was significantly ($P \leq 0.05$) positively correlated to disease severity at early milk (GS 77) ($r = 0.366^*$) and at soft dough/final disease severity (GS

Table 16: Correlation relationship between disease severity, epidemiological and yield parameters on wheat crop

	Severity at GS 55	Severity at GS 65	Severity at GS 77	Severity at GS 85	AUDPC	Yield (t/ha)	Test weight (g)
Severity at GS 55	1	.226	-.024	.197	.253	-.173	.117
Severity at GS 65		1	.366*	.424*	.592**	-.535**	-.374*
Severity at GS 77			1	.811**	.869**	-.760**	-.782**
Severity at GS 85				1	.957**	-.905**	-.721**
AUDPC					1	-.915**	-.754**
Yield (t/ha)						1	.724**
Test weight (g)							1

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

85) (r=0.424*). This implies that a disease severity at GS 65 will have a significant impact to disease progress on subsequent stages of wheat crop. Disease severity at flowering (GS 65) was highly significant (P≤0.01) and positively correlated to AUDPC. Thus an infection at GS 65 will greatly affect the AUDPC and thus the rate of infection on wheat stem rust on the crop. There was a highly negative correlation (P≤0.01) of disease severity at flowering with the grain yield (r=-0.535**), implying that disease infection at this stage is very critical in reducing the grain yield. Although a significant negative correlation (r=-0.374*) was also reported on 1000 kernel weight (P≤0.05), this was not as high as that of grain yield (P≤0.01), perhaps due to the fact that

the seeds have not yet fully formed at this stage, and thus no much impact on their quality at this stage.

5.3.4.6.3 Correlation coefficient relationship of disease severity at GS 77 (early milk)

Disease severity at early milk (GS 77) was highly significantly ($P \leq 0.01$) positively correlated to disease severity at soft dough stage/final disease severity (GS 88) ($r = 0.811^{**}$). This implies that a disease severity at GS 77 will have a significant impact on the progress of the disease in the subsequent stages of wheat crop development. Disease severity at milk stage (GS 77) was highly significant ($P \leq 0.01$) and positively correlated to AUDPC ($r=0.869^{**}$). Thus an infection at GS 77 will greatly affect the AUDPC and thus the rate of infection on wheat crop. There was a highly negative correlation ($P \leq 0.01$) of disease severity at milk stage with the grain yield ($r=-0.760^{**}$), implying that disease infection at this stage is very critical in reducing the grain yield. At this stage (GS 77) there was a highly significant ($P \leq 0.01$) negative correlation on test weight ($r=-0.782^{**}$), thus implying how this growth stage (GS77) is very critical in affecting the test weight. Although correlation studies of wheat stem rust against yield at different growth stages have not been reported in Kenya, Macharia, (2009) shows there exist a significant negative correlation of final disease severity (GS 77) to wheat yield components.

5.3.4.6.4 Correlation coefficient relationship of AUDPC on grain yield and test weight

The area under disease progress curve (AUDPC) was highly significantly ($P \leq 0.01$) negatively correlated to the grain yield ($r=-0.915$). Thus implying the importance of AUDPC in determining the grain yield. A highly significant negative correlation was observed between AUDPC and the test weight ($r=-0.754^{**}$) thus implying also that AUDPC is very critical in determining the test weight. Similar trend of AUDPC on grain yield and test weight have been reported by Macharia, (2009).

CHAPTER SIX

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

In a span of 10 years three virulent races of wheat stem rust have been identified (Jin *et al.*, 2009), the grain losses associated with these races is currently estimated at 70-100% (Wanyera *et al.*, 2008a). Apparently all these races have been identified in Kenya, and its highly suspected that the environment in East Africa offers a conducive environment for evolution of these new races. Uasin gishu region is considered to be the grain basket of Kenya producing up to 25% of our wheat crop (MoA, 2010). From this study, it was clear that wheat stem rust is wide spread in this region, the spread of wheat stem rust is influenced by the altitude, which generally affect the temperature and humidity levels. Roelf *et al.* (1992) showed that wheat stem rust is severe when both temperatures and relative humidity are high. The high stem rust severity in places like Mois bridge is a reflection of this. Singh *et al.* (2006) proposed that evolution of these new races could be attributed to high inoculum pressure, thus increasing chances of mutation. From this study a new race PTKST was identified in areas with high stem rust severity, this is of critical concern as it shows why we need to reduce the inoculum pressure either through breeding or by use of fungicides. It was also noted that there has been a shift in predominant race from Ug99 (TTKSK) in 2005 (Wanyera *et al.*, 2006), to currently TTKST. Shift of predominant race has been reported also in other countries like Ethiopia (Belayneh *et al.*, 2008) and USA (Jin, 2005), this is mainly attributed to the varieties being grown at that time. From this study, the predominant race TTKST was highly suspected to be linked to the common variety grown in this region which has *Sr 24* gene that this race is virulent to.

Pretorius *et al.* (2000) reported a virulent race Ug99 (TTKST), this race was reported to be virulent to be virulent to 29 of the 46 monogenic wheat stem rust differentials (Jin *et al.*, 2007). The current study has shown that the new Ug99 mutants races are virulent to some of 17 monogenic lines which were previously resistant. Race TTKST which was discovered in Kenya (Jin *et al.*, 2008) was found to be the most virulent race in this study. Such findings concur with Pretorius *et al.* (2010), who suggested that races which are virulent to *Sr 24* gene are more

virulent than the original Ug99. The depletion of these novel sources of resistance calls for more effort in finding other sources of resistance.

Fungicides will continue to play an important role in control of these virulent races of wheat until varieties with adequate level of resistance are released (Wanyera, 2008b). The new races have lead to increased cost of control due to poor choice of fungicides, rates and proper timings (GRI, 2006). Silvacur 375g/L was found to effectively control wheat stem rust. This study found out that its possible to effectively control wheat stem rust under a single spray regime as long as proper rates and timings are observed. These findings will go along way in helping farmers reduce the cost of production, by adopting a single spray regime and appropriate rates.

6.2 Conclusions and recommendations

Wheat stem rust is severe and widespread in Uasin Gishu. The predominant race in this region is race TTKST, a new race PTKST was also reported. These two races are virulent to *Sr 24* gene, which is present in the commonly grown variety in this region.

The study therefore recommends that:

1. Similar studies should be carried out in other major wheat growing areas of Kenya with emphasis on, effect of agro ecological zone on stem rust severity and diversity in commonly grown varieties. Furthermore the effect of cultural practices employed on the race dynamics and cause of these virulent mutations should be investigated.
2. The genetic inheritance of the new race PTKST need to be studies to determine if it is a mutant of race Ug99 or an introduced race.
3. The four races identified in this study should be tested on all previously resistant wheat materials.

The study on screening the virulence of the four races identified on the previously resistant materials concluded that race TTKST and race PTKST are virulent to some of the previously resistant wheat differentials. A combination of these two races showed virulence on all the differentials tested. Therefore races virulent to *Sr 24* gene were found to be more virulent than the original Ug 99 and its mutant TTTSK.

The study therefore recommends that:

1. Intensified search for other sources of resistance, especially those resistant to the virulent race Ug99 and its mutants, be undertaken.
2. With the depletion of the existing source of resistant materials, mutation breeding should be adopted to try and develop new resistant genes and lines.

The study on wheat stem rust control using Tebuconazole 250g/L + Tridimenal 125 g/L revealed that it is possible to effectively control wheat stem rust using a single spray regime, as long as proper rates and timings are adopted. The low fungicide rate of 0.75 L/ha was effective only during the short rain season (October-January) when the disease pressure was low. The timing of application should be at 10%. Therefore, a farmer can minimize the use of chemicals during the short rain season. It was evident that during the long rain season a single dose of 0.75 L/ha did not give the best level of control and consequently yields. When the disease pressure was high (long rain season of May-September) a rate of 1 L/ha applied at 10%.

The study therefore recommends that:

1. Similar studies be carried out on all fungicides, both new and those currently recommended for wheat stem rust control.
2. Economic analysis studies be carried out to advice the farmers on the pros and cons of using fungicides.
3. A combination of similar studies with those varieties that are thought to contain some useful resistance genes like the adult plant resistant gene (*Sr 2* complex) be conducted.

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APPENDICES

Appendix 1: Races of stem rust recorded in Kenya from 1928 to 2008

Race		
Kenya No.	**International No.**	**Year Identified**
K1	21	1928
K2	17	1928
K3	34	1930
K4	116	1931
K5	21	1936
K6	107	1940
K7	Not given	1943
K8	24	1948
K9,10,11	122,24,24	1950
K12	21	1951
K13,14	Not given	1953
K15	Not given	1954
K16	Not given	1955
K17,18,19	Not given	1956/57
K20	Not given	1958
PgtUg99	TTKSK	2003
Sr31+Sr24	TTTST	2007
Sr31+Sr36	TTTSK	2008

Appendix 2: Anova for Final Disease Score (FDS)

Source	d.f	m.s	v.r	F pr
Season	1	102.4	0.88	0.446
Rate	4	12808.07	372.63	<.001
Season. Rates	4	10.18	0.3	0.876
Time	2	634.01	28.51	<.001
Season. Time	2	94.9	4.27	0.021
Rate. Time	8	136.23	6.13	<.001
Season. Rate. Time	8	66.01	2.97	0.01
Rep	2	22.18	0.19	
Error	40	22.24		
c.v	12.6%			
P=0.05				

Appendix 3: Anova for AUDPC

Source	d.f	m.s	v.r	F pr
Season	1	220572	3.36	0.072
Rate	4	5796755	88.18	<0.001
Season. Rates	4	220777	3.36	0.015
Time	2	480343	7.13	0.001
Season. Time	2	97659	1.49	0.235
Rate. Time	8	67531	1.03	0.426
Season. Rate. Time	8	29567	0.45	0.886
Rep	2	155580	2.37	
Error	58	65738		
c.v				
P=0.05				

Appendix 4: Anova for 1000 kernel weight

Source	d.f	m.s	v.r	F pr
Season	1	3.173	3.51	0.202
Rate	4	259.649	103.28	<0.001
Season. Rates	4	12.331	4.91	0.009
Time	2	8.157	3.50	0.04
Season. Time	2	3.238	1.39	0.261
Rate. Time	8	3.804	1.63	0.147
Season. Rate. Time	8	2.645	1.13	0.362
Rep	2	0.502	0.57	
Error	40	2.332		
c.v				
P=0.05				

Appendix 5: Rainfall and temperature data recorded during the experiment period (2009 - 2010) at KARI Njoro

Year	Month	Rainfall (mm)	Maximum (^{o}C)	Minimum (^{o}C)
2009	January	21.7	25.0	9.0
	February	5.7	27.0	8.0
	March	24.8	28.0	9.0
	April	62.7	26.0	9.0
	May	173.8	24.0	9.0
	June	13.6	24.0	8.0
	July	42.2	23.0	7.0

	August	56.3	25.0	9.0
	September	45.1	25.0	8.0
	October	74.8	22.0	10.0
	November	62.2	23.0	9.0
	December	76.7	23.0	10.0
2010	January	42.9	23.3	9.0
	February	157	25.0	10.5
	March	184.1	23.0	10.0
	April	140.4	23.0	10.0
	May	180.8	22.0	11.0
	June	51.8	22.0	9.0
	July	166.1	21.0	9.0
	August	240	21.0	9.0
	September	172.2	23.0	8.0
	October	109.9	22.0	10.0
	November	55.4	23.0	10.5

Appendix 6: Growth stages of small grains

CODE	Description	CODE	Description
0	**GERMINATION**	37	Flag leaf just visible
00	Dry seed	38	Flag leaf ligule just visible
01	Start of imbibitions	**4**	**BOOTING**
03	Imbibition complete	41	Flag leaf sheath extending
05	Radicle emerged from seed	43	Boots just visible swollen
07	Coleoptile emerged from seed	45	Boots swollen
09	Leaf just at coleoptiles tip	47	Flag leaf sheath opening
1	**SEEDLING GROWTH**	49	First awns visible
10	1st leaf through coleoptiles	**5**	**EAR EMERGENCE**
11	1st leaf unfolded	51	1st spikelet of ear emerged
12	2 leaves unfolded	53	One-fourth of ear emerged
13	3 leaves unfolded	55	One-half of ear emerged
14	4 leaves unfolded	57	Three-fourths of ear emerged
15	5 leaves unfolded	59	Emergence of ear completed
16	6 leaves unfolded	**6**	**FLOWERING**
17	7 leaves unfolded	61	Beginning of flowering
18	8 leaves unfolded	65	Flowering half-way complete
19	9 leaves unfolded	69	Flowering complete
2	**TILLERING**	**7**	**MILK DEVELOPMENT**
20	Main shoot only	71	Seed water ripe
21	Main shoot and 1 tiller	73	Early milk
22	Main shoot and 2 tillers	75	Medium milk (An increase in the

			solids of the liquid of the endosperm is notable when crushing the seed between fingers)
23	**Main shoot and 3 tillers**	**77**	**Late milk**
24	Main shoot and 4 tillers	**8**	**DOUGH DEVELOPMENT**
25	**Main shoot and 5 tillers**	**83**	**Early dough**
26	Main shoot and 6 tillers	85	**Soft dough (Fingernail impression not held)**
27	**Main shoot and 7 tillers**	**87**	**Hard dough (Fingernail impression not held; head losing chlorophyll)**
28	Main shoot and 8 tillers	**9**	**RIPENING**
29	**Main shoot and 9 tillers**	91	**Seed hard (difficult to divide by thumbnail)**
3	**STEM ELONGATION**	92	**Seed hard (can no longer be dented by thumbnail)**
30	Pseudostem erection	93	Seed loosening in daytime
31	**1st node detectable**	**94**	**Over-ripe; straw dead/ collapsing**
32	2nd node detectable	95	Seed dormant
33	**3rd node detectable**	**96**	**Visible seed giving 50% germination**
34	4th node detectable	97	Seed not dormant
35	**5th node detectable**	**98**	**Secondary dormancy induced**
36	6th node detectable	99	Secondary dormancy lost

(Adopted from Zadok *et al.*, 1974)