Stem rust resistance in South African wheat and triticale

by

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Declaration

I, Cornelia Magrietha Bender, declare that the thesis that I herewith submit for the Doctoral Degree in Plant Pathology at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

18 March 2020

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Date

Dedication

This thesis is dedicated to my mother, Johanna and my late father Bertus Barnard who triggered in me a never-ending curiosity in nature. In addition, I am indebted to my husband Johan and boys Francois and AJ who have always been my source of inspiration.

Look deep into nature and
then you will understand
everything better

Albert Einstein

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Picture credits: Rust Laboratory, University of the Free State.

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List of Abbreviations

AKA	acrylamide:bis-acrylmide
AP	appressorium
APR	adult plant resistance
ARC	Agricultural Research Council
ASR	all-stage resistance
B.C.	before Christ
BGRI	Borlaug Global Rust Initiative
CI(‘s)	coefficient of infection(s)
CIM	composite interval mapping
CIMMYT	International Maize and Wheat Improvement Centre
cm	centimetre(s)
CO_2	carbon dioxide
CTAB	hexadecyltrimethylammonium bromide
DH	doubled haploid
DNA	deoxyribonucleic acid
dpi	days post-inoculation
e.g.	*exempli gratia* (for example)
EDTA	ethylene-diamine-tetraacetic acid
ESWYT	Elite Spring Wheat Yield Trial
et al.	*et alii* (and others)
f. sp.	*forma specialis*
FAO	Food and Agriculture Organization of the United Nations
FITC	fluorescein isothiocyanate conjugate
g	gram(s)
g	relative centrifugal force
CGIAR	Consultative Group on International Agricultural Research
GT	germ tube
h	hour(s)
ha	hectare(s)
HCL	hydrochloric acid
HCN	host cell necrosis
HMC	haustorial mother cell(s)
hpi	hours post inoculation
ICARDA	International Center for Agricultural Research in the Dry Areas
IT	infection type
ISRTN	International Stem Rust Trap Nursery
KOH	potassium hydroxide
kg	kilogram(s)
kPa	kilopascal
L	litre(s)
Ltn	leaf tip necrosis
Lr	leaf rust resistance gene
m	metre(s)
M	molar
MAS	marker-assisted selection
mg	milligram(s)
min	minute(s)

mL	millilitre(s)
mM	millimolar
mol	mole
MR	moderately resistance
MS	moderately susceptibility
MSL	Marker Service Laboratory
NA	North American
nm	nanometre(s)
NPK	nitrogen, phosphorus and potassium
P	*Puccinia*
Pg	*Puccinia graminis*
Pgt	*Puccinia graminis* f. sp. *tritici*
pH	potential hydrogen
PIH	primary infection hypha
QTL	quantitative trait loci
R	resistance
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
S	septum
S	susceptibility
SA	South Africa
SCAR	sequence characterized amplified region
SEM	scanning electron microscopy
SIH	secondary infection hypha
SIT(s)	seedling infection type(s)
SNP	single nucleotide polymorphism
spp.	species
Sr	stem rust resistance gene
SSR	simple sequence repeat
SSV	substomatal vesicle
STS	sequence tagged sites
subsp.	subspecies
T	trace
T.	*Triticum*
TBE	Tris-HCl/Borate/EDTA
TEMED	N,N,N',N-tetramethylethylenediamine
UK	United Kingdom
US	urediniospore
USA	United States of America
UVPgt	Universiteit Vrystaat *Puccinia graminis* f. sp. *tritici*
v	volume
viz.	*videlicet*
w	weight
WGA	wheat germ agglutinin
Yr	stripe rust resistance gene
µg	microgram(s)
µL	microliter(s)
µm	micrometre(s)
%	percentage
°C	degrees Celsius

GENERAL INTRODUCTION

Wheat (*Triticum aestivum* L.), grown on more than 218 million ha worldwide, is a staple food source for around 40% of the world's population. The crop is believed to have originated in the Near East in areas known today as Syria, Turkey, Afghanistan, Iraq and Iran with archaeological remains of domesticated wheat dating back to 5 500 BC in Anatolia, Turkey. The cultivation of wheat expanded from the Fertile Crescent to the rest of the world, including South Africa (SA) where the first wheat was planted by Dutch settlers soon after their arrival on 16 April 1652. Over the years the area under wheat cultivation has increased and production is currently ranked second after maize.

Rusts have threatened wheat production since early agriculture and epidemics were so vivid that religious ceremonies were held to appease Robigo, the rust god. This became an annual festival called Robigalia, celebrated on 25 April and was later incorporated into Christianity as the St. Marks Day or Rogation. Stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is the most damaging of the three rusts, because it infects not only the leaf sheath, peduncles and stems, but also the leaves and spikes. Epidemics, whether large or small scale have been observed in nearly every country where wheat is grown. The first wheat stem rust epidemic was documented in 1726 in the Western Cape and since then yield losses have often been recorded in South Africa. Eighteen different pathotypes were identified from surveys between the 1920s and 1973 and to date 32 pathotypes have been described. In surveys conducted during 2018, stem rust severities of up to 30% were observed in the Western Cape, emphasizing the continued importance of the disease.

In SA the breeding of triticale (X *Triticosecale* Wittmack), an amphiploid species carrying both wheat and rye genomes, was initiated in 1960 at the University of Stellenbosch and in 1975 three tall lines were released to farmers. Since then triticale production has increased and currently approximately 55 000 ha are planted annually, mostly in the Western Cape, either as a cover crop in vineyards or as a feed grain. Soon after commercial triticale production started, *Pgt* emerged

as a problem and with various *Pgt* pathotypes documented on triticales, the vulnerability of this crop became evident when a new pathotype rendered most of the cultivars susceptible in 2005.

Control of wheat rusts in SA relies mostly on the use of genetic resistance and fungicide applications. Although it is possible to minimize losses by the timely applications of fungicides this is costly thereof is not financially viable in the long-term, especially under dryland conditions, where the yield potential can be severely affected during low rainfall seasons. In addition, fungicide application is not environmental friendly, poor timing of applications add to production risk and long-term usage can result in fungicide resistance. Other control measures include cultural practices such as changing sowing dates, use of early maturing cultivars, multi-lines or varietal mixtures and destroying of volunteer plants or alternate hosts. None of these strategies are currently followed in SA. The incorporation of resistance genes into commercial cultivars by breeders and the cultivation of resistant cultivars is an environmental friendly and more profitable way of stem rust control. Three major factors that need consideration in stem rust control include 1) possible yield loss due the disease, 2) the occurrence of new, more virulent pathotypes and 3) effectiveness and cost of control measures.

To ensure profitability for local wheat producers, cultivar selection is a critical decision in input cost and risk management. The deployment and production of resistant cultivars can help to maximize yields and to minimize the risk of stem rust epidemics. Since stem rust occurs almost wherever wheat is grown, with potential to cause severe damage, planting of well-characterized cultivars is important and allows wheat producers to optimize preventative control measures in high-risk areas. Therefore, the long-term evaluation of wheat varieties that include both commercially available cultivars and advanced breeding lines for stem rust resistance provides fundamental information in plant protection strategies for breeders and producers.

Resistance to stem rust is broadly classified as all stage resistance (ASR), which can be detected in wheat seedlings and all subsequent growth stages, and adult

plant resistance (APR), which is most effective in mature plants in the field. Since APR is a priority in most stem rust resistance breeding programs, accurate assessment thereof is essential for developing varieties carrying this type of resistance. The efficiency of stem rust phenotyping can be improved by scoring adult plant responses in a greenhouse compared to costly, seasonal restricted and time consuming field trials. There is need for field validation due to interaction effects that cannot be observed in the greenhouse. Benefits of such a greenhouse method include control of the racial identity of inoculum and the uniformity of application and it can provide reliable and relatively rapid results. Although it accelerates phenotyping of stem rust resistance in breeding it should also accurately reflect field data.

Resistance to stem rust has often been based on ASR genes worldwide. Likewise, the historic deployment of major genes against stem rust has resulted in a narrow genetic base of resistance in SA and many of these single genes have failed due to the evolution of new pathogen races. To stay ahead of an evolving pathogen, there is a constant need to discover new sources of resistance, understand the genetic base of presently deployed sources in cultivars, and to manipulate the future deployment of resistant sources through a more sustainable approach. Therefore, genetic studies that include seedling analysis to detect the presence of ASR and adult plant trials to detect APR in selected SA wheat and triticale cultivars through inheritance studies are essential and can assist breeders to discern between monogenic and more complex resistance sources.

To add to our understanding of the host-pathogen interaction, which is the basis of disease phenotype observed as signs and symptoms, histological investigations elucidate the infection structures and subsequent colonisation process of rust pathogens in their cereal hosts. According to available literature, no comprehensive information exists for the infection process of *Pgt* on triticale and whether infection structure differentiation in this pathosystem differs from other small grain hosts.

The objectives of the current study were:

- To characterize South African wheat varieties for seedling and adult plant stem rust resistance through multi-season greenhouse and field trials and to determine the impact of new, virulent races.
- To develop a dependable screening system for assessing APR for stem rust under greenhouse conditions and to verify this method using a diverse wheat germplasm pool.
- To analyze the genetics of stem rust resistance in the South African spring wheat cultivars Duzi, SST 047, Steenbras, Krokodil, Tankwa and Betta.
- To assess the infection process and nature of resistance in the South African triticale cultivars Kiewiet and Tobie.

Chapter 1: Literature review

INTRODUCTION

Cereals, which include wheat, barley, rye, oats, rice, maize and millet are the most important food source for human consumption and, with a projected world population of 10 billion in 2057 (accessed November 2019 http://www.worldometers.info/world-population/#growthrate), annual cereal production needs to grow by more than 40 percent (FAO, 2009). Wheat is grown on more hectares than any other cereal and is one of the most important sources of calories for humans (Crop Prospects and Food Situation (FAO) #2, July 2019). However, the growth rate of wheat yields has declined since the Green Revolution years. It dropped from 6% in the 1960s to 2% in the 1990s (accessed February 2020 http://www.fao.org/3/y3557e/y3557e08.htm). Therefore, it is essential to increase global wheat production which will require two potential approaches: 1) agricultural extensification and / or intensification. The extent to which agricultural extensification can be applied is restricted because of limited arable land for crop production. However, 2) sustainable intensification has been considered the best component of action to achieve food security (Charles, Godfray and Garnett 2014) and the goal thereof is to achieve higher yields with less harmful impacts on the environment, which can be attained by making use of existing resource, such as species and genetic biodiversity, efficiently. To achieve this, global consortiums such as the Borlaug Global Rust Initiative (BGRI), the Consultative Group on International Agricultural Research (CGIAR), which include the International Center for Agricultural Research in the Dry Areas (ICARDA) and International Maize and Wheat Improvement Centre (CIMMYT) are assisting wheat breeding institutions and producers. This illustrates global collaboration that is needed for agricultural progression worldwide.

Numerous biotic and abiotic stresses affect wheat production even in the era of genetically improved varieties. Abiotic stresses such as soil salinity, pollutants, radiation and global climate change which influence temperature, precipitation and length of growing season are limiting productivity and might modify the impact of biotic stresses such as pests, diseases and weeds. The establishment of healthy

plants both early and during wheat production is generally viewed as essential. Some of the most important biotic stresses are the rust fungi causing stem, leaf and stripe rust of wheat. According to Chamy (2014) Dr David Hodson of CIMMYT said in Addis Ababa that the disease's risk lies in "its ability to cause large-scale destruction in a very short period of time over very large cultivated areas". Currently wheat stem rust caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn (*Pgt*), once the most feared disease of bread wheat (*Triticum aestivum*), has re-emerged as an economically important disease (Singh *et al*., 2015).

WHEAT

The exact origin of wheat *(T. aestivum* L. em Thell.) as a cultivated crop is still unknown. Wheat most likely evolved from wild grasses in the region known in early history as the Fertile Crescent, an area of the Tigris-Euphrates drainage basin in the Near East. Wheat is important in agriculture, primarily as a food crop and an economic commodity and is currently the most widely consumed cereal after rice. According to www.economist.com (accessed July 2019) wheat consumption is rising quickly in Asian countries of which most has to be imported. Total world wheat production for 2017/18 was 761 million tons, whereas for 2018/19 an estimated global decrease of 3.7% could lead to 28 million tons less being produced (www.worldwheatproduction.com). The European Union is leading by producing about 20% of the world's wheat stock, followed by China, India, Russia and the USA with South Africa ranked around 27th globally. The first wheat was planted in South Africa during the winter of 1652, whereas the first breeding program was established in 1891 (Du Plessis, 1933).

Globally the highest levels of hunger occur in Sub-Saharan Africa and in South Asia. Although the Global Hunger Index declined in South Africa from 18.5 in 1992 to 14 in 2019 (accessed November 2019 www.globalhungerindex.org), the situation remains alarming. The demand for wheat continues in SA and even though 1.7 million tons was imported during the 2019 marketing season, wheat is still one of the main grain crops cultivated. The area of 503 350 ha planted slightly increased during the 2018/2019 season with an improved average production of 1 868 000 tons (accessed 12 November 2019 www.grainsa.co.za). The rise in yield per

hectare was due to increased efficiency, productivity and quality. There are three different wheat producing areas making the production of wheat quite unique. Two dryland areas are used for wheat production, one of which is winter wheat grown on stored moisture from the previous summer and autumn in the Free State, and, the second a region in the Mediterranean climate of the Western Cape. Irrigated spring wheat is grown in the summer rainfall area where sufficient water supplies are available (Van Niekerk, 2001). The Western Cape region, which includes Namaqualand, the Swartland, and the Rûens, is the main production area in South Africa contributing almost 50% of the annual wheat crop (www.grainsa.co.za/pages/industry-reports/production-reports) (South African Grain Laboratory Wheat Report 2018/2019). Spring wheat is planted in autumn because the Western Cape's winter rainfall provides enough soil moisture for germination and it protects seedlings from high soil temperatures expected during prevailing summers (Curtis, 2002).

Increasing local wheat production will either require an increase in arable farming land for wheat production in South Africa, that is not viable therefore, intensification is considered the best method to achieve higher yields. This can be achieved by better utilizing of available genetic biodiversity, improved agronomic practices and selection of crop varieties together with lower input costs. Cultivar choice is an essential means to optimize yields and to manage risks. Agronomic characteristics such as adaptability, yield potential and disease resistance of commercial cultivars should be taken into consideration, and, if executed correctly, optimal grain yield can be achieved.

TRITICALE

Triticale (*xTriticosecale* Wittmack ex A. Camus; 2n = 6 × = 42) is an amphiploid man-made species carrying the genomes of wheat (*Triticum* spp.) and rye (*Secale cereale*). Most triticales are progenies of either common wheat (*Triticum aestivum* L., 2n=42=AABBDD) or durum wheat (*Triticum durum*, 2n=28=AABB) as female parent and diploid rye (*Secale cereale* L., 2n=14=RR) as male parent (Ammar *et al.*, 2004). Triticale belongs to the family *Poaceae*, subfamily *Pooideae* and tribe *Triticeae* (Kavanagh *et al.*, 2010). Triticale is an important crop because of its stable

performance in less productive environments such as abiotic stress conditions in soil, including aluminum toxicity, salinated or acid soil, waterlogged soils and drought (Kuleung *et al.*, 2004, McGoverin *et al.*, 2011, Oettler, 2005). In general triticale combines the good grain quality and yield potential of wheat with disease and environmental tolerance (above mentioned soil factors) of rye. Triticale resembles either wheat or rye and although it is generally grown for forage or fodder, some triticale-based foods are available at health food stores. Triticale flour is used for bread, pizza dough, cookies, pasta and breakfast cereals, whereas kernels are used for malting, brewing and animal feed (grain, forage, grazing, silage, hay and straw) (McGoverin *et al.*, 2011). Another potential us of the crop is as a source of bio-energy and bio-ethanol (Klickocka *et al*., 2019, McGoverin *et al.,* 2011).

Triticale varieties can be classified into winter, intermediate or spring types of which the latter have a short growth period, are day length insensitive and exhibit upright growth with lavish forage and are therefore mostly cultivated in warmer areas such as SA and Australia (Santiveri *et al.*, 2002, Mergoum *et al.*, 2009, Salmon *et al.*, 2004). In SA, spring triticale has been produced since 1970 (Littlejohn *et al*., 1991). Currently more than 55 000 ha are planted in SA per year which is mainly used as animal feedstock (50%), as silage (25%) and in vineyards as a cover crop (25%) (Roux *et al.*, 2006, Boshoff *et al*., 2019). Most of these production areas are located in the Western Cape where environmental factors, such as temperature and abundant moisture, favour diseases. Diseases were initially not a serious problem, however as production area increased most wheat and rye diseases surfaced on triticale (Zillinsky, 1985, Singh and Saari, 1991, Mergoum, 1994).

THE STEM RUST PATHOGEN

History, distribution and importance

Wheat rust diseases (stem, leaf and stripe) caused by *Puccinia spp*. are among the oldest plant diseases known to humans and affect wheat production globally. Wheat leaf fragments infected with stem rust were found in a storage jar from the Late Bronze Age (Kislev, 1982). Pliny the Elder reported that the festival of Robigalia, (“to appease the fertility god Robigus, god of rusts and mildews”) was introduced around 700 BC during the reign of the second Roman king Numa Pompilius (Pliny

69). The general agreement is that the center of origin of pathogens is usually the same as the center of origin of the host species, in this case the Fertile Crescent, where rusts were a constant problem on wheat possibly since its domestication about 8 000 years ago. The rusts plagued farmers throughout history, but it only became known that they were caused by fungi in the 19th century. The earliest detailed reports were individually given by the Italian scientists Fontana and Tozzetti in 1767 (translated and published by the AmericanPhytopathological Society as Fontana, 1932, Tozzetti, 1952). Stem rust was named by Persoon in 1797 as *Puccinia graminis* (Schafer *et al.*, 1984), whereas Stakman and Piemeisel (1917) were the first to demonstrate that the stem rust pathogen had various physiological races/pathotypes within the wheat attacking *forma specialis* of *Pg*.

Stem rust is a devastating disease, causing losses of 50% under favourable conditions and when combined with susceptible varieties, losses of up to 100% can occur. However, epidemics vary in magnitude and frequency depending on host genotype and environment, with wet years considered to be ‘rust’ years. Periodically damaging epidemics have plagued humanity when crop failures due to stem rust were reported as early as the 1700’s by the Jesuit colony in Rio Grande do Sul, Brazil. Other early reports include Australia and Spain where stem rust epidemics were recorded in 1795 (White, 1981) and 1877 (Ruiz de Casaviella, 1878).

In North America the first reported epidemic occurred in spring wheat in 1878 (Hamilton, 1939). Thereafter, epidemics occurred in 1904, 1909, 1916, 1919, 1923, 1925, 1927, 1929, 1937, 1938, 1955, 1986 and 1991 with severe epidemics in 1935, 1953 and 1954 (Roelfs, 1978). Crop losses of up to 50% were recorded in North Dakota and Minnesota and 20% or more in South Dakota during the most severe epidemics in North America (Leonard, 2001). Subsequently, significant losses due to stem rust have been reported (Dean *et al.*, 2012, Singh *et al.*, 2015) and in Ethiopia major losses of 42% in 1993-94 and an almost 100% yield loss on the susceptible cultivar Digalu at the beginning of 2014 were reported (Dubin and Brennan, 2009, Olivera *et al*., 2015).

According to Pretorius *et al.* (2007) the first wheat stem rust epidemic was documented in SA in the Western Cape in 1726. Thereafter, stem rust was regularly associated with wheat production and epidemics were recorded in the winter rainfall areas of the Western and Eastern Cape (Le Roux, 1989, Le Roux and Rijkenberg, 1987) and yield losses of over 35% in the summer-rainfall regions of the Free State (Pretorius *et al.*, 2007). These main production areas in South Africa remain favourable for rust development, qualifying stem rust as an important production constraint. Furthermore, it is widely distributed and remains viable after dispersal over vast distances (Visser *et al*., 2019). The pathogen's capacity to mutate for virulence on previously resistant varieties and the rapid rate of disease increase qualifies stem rust as one of the most feared diseases of wheat worldwide.

Symptoms and signs

Stem rust of wheat, caused by *Pgt*, gets its name from its appearance on the plant, where infection occurs mostly on stems, but can occur on any above-ground plant part (Fig 1.1 A, B and C). Pustules become visible as early as five days after infection (containing thousands of dry reddish-brown spores) looking literally like "rust" on the plant. Stem rust is also known as black rust, because as a result of unfavourable conditions or at the end of the season, shiny black teliospores are produced in the uredinium (Fig 1.1 D). Oblong pustules (uredinia) occur primarily on stems but can also be found on leaves, sheaths, glumes, awns, and occasionally seed (Leonard and Szabo, 2005). Signs commence as oval to elongate lesions turning reddish-brown as soon as spores mature and can measure up to 3 x 10 mm in dimensions. Mature pustules release urediniospores which are ovoid in shape, measuring 15-20 and 40-60 µm (Wiese, 1987). Pustules on the leaves are visible on both sides of the leaf. At close observation of the pustules, pieces of shredded epidermis are visible at their edges (Fig 1.2 A). On the stems pustules are elongated (Fig 1.2 B, C and D) and coalesce to cover large areas, especially with severe infections, resulting in the collapse of stems with heavy rain or wind or it interrupts nutrient flow to developing heads. Lodging impedes mechanical harvesting, causing major yield losses. Furthermore, *Pgt* absorbs nutrients essential for plant growth and with the loss of water, through the broken epidermis associated with pustules, the plant's metabolism becomes less efficient leading to shriveled grain (Schumann and Leonard, 2000).

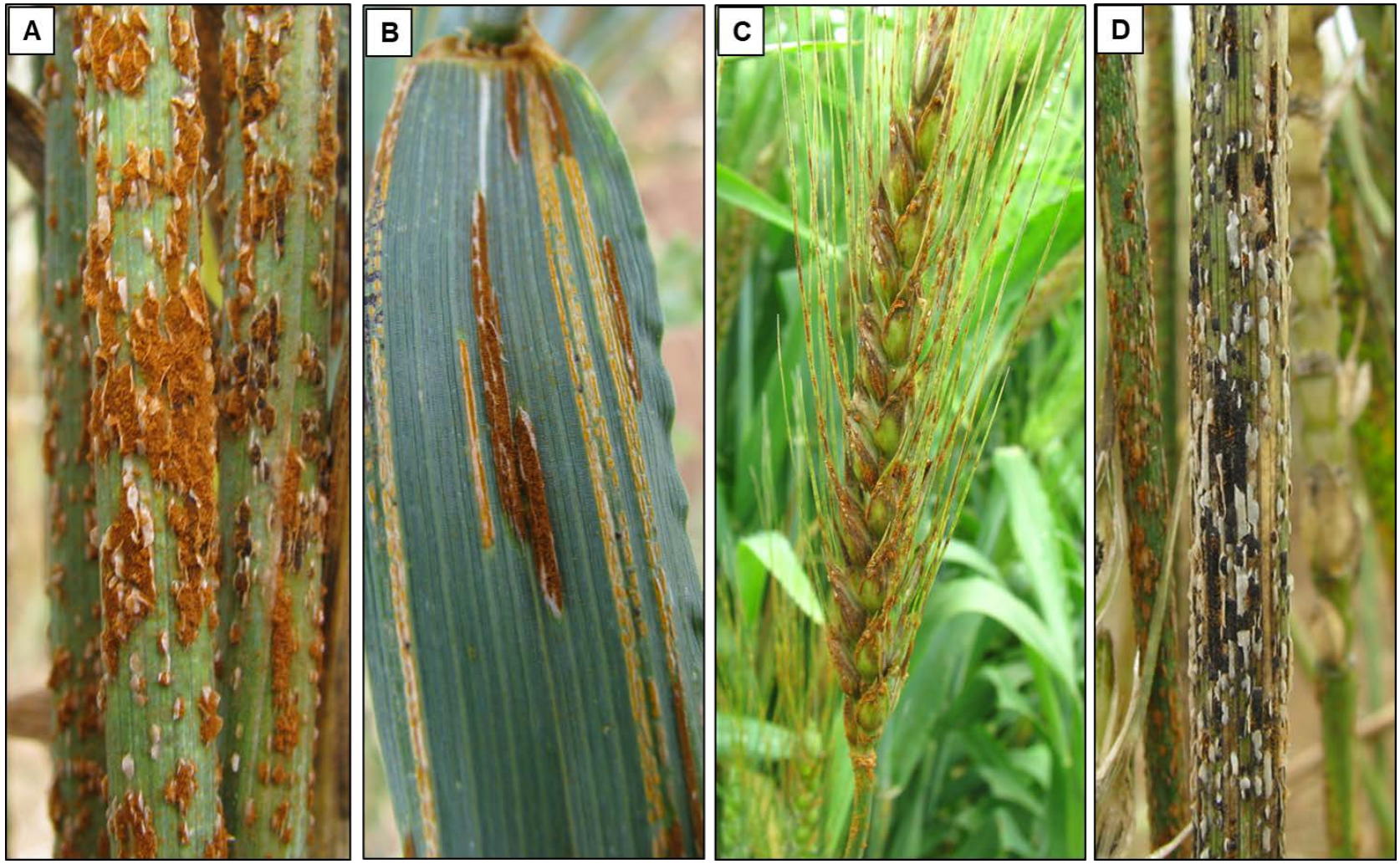

Figure 1.1: Wheat stem rust uredinia on (A) stems, (B) flag leaf (with accompanying stripe rust) and (C) spike. Telia, containing teliospores, are shown in (D).

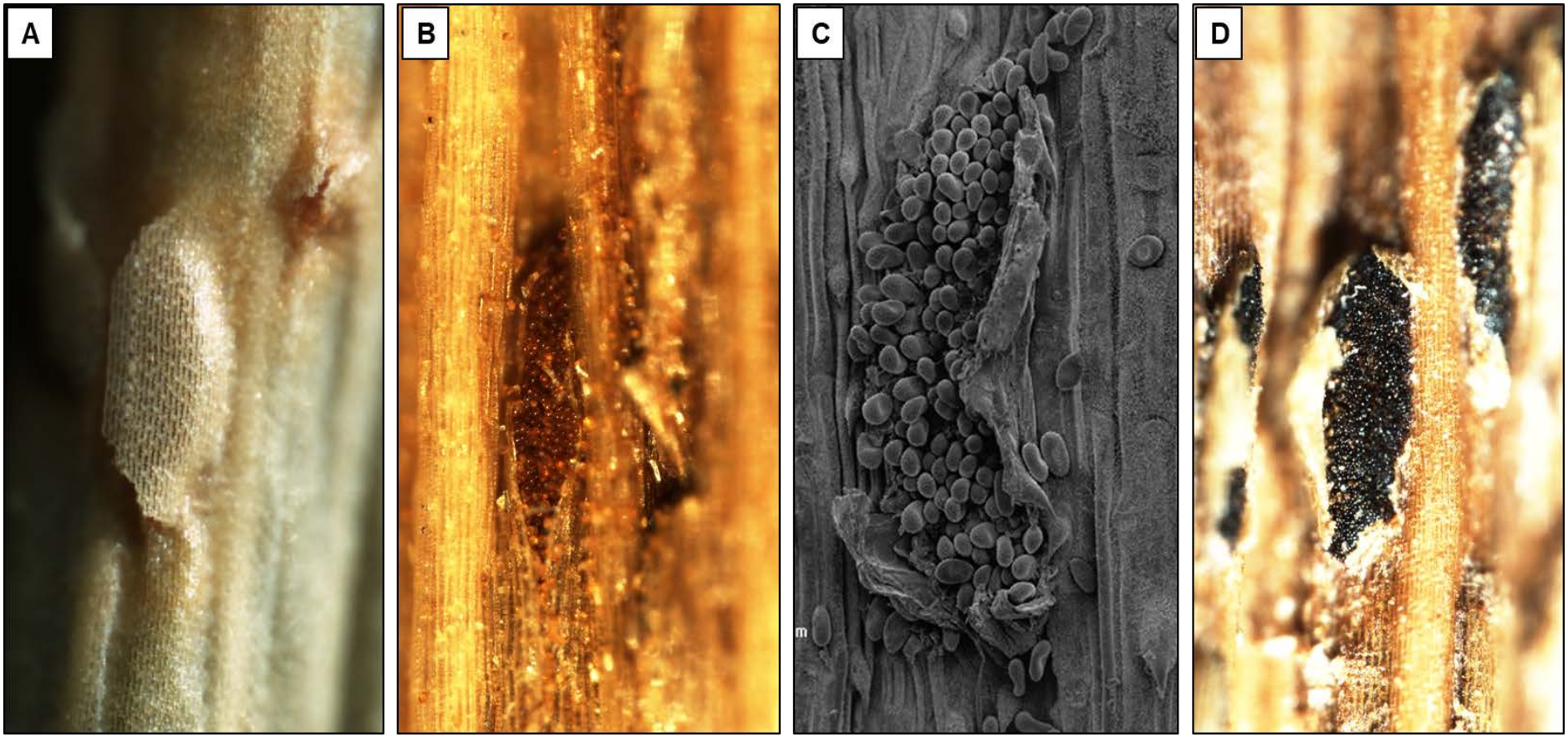

Figure 1.2: Stem rust pustules on wheat stems with (A) shredded epidermis, (B) and (C) urediniospores, and (D) teliospores.

Epidemiology and infection process

The *Puccinia* spp. are obligate parasites, also called biotrophs and therefore need living wheat plants or other secondary hosts for survival (Singh, 2002, Dadkhodaie *et al.*, 2011). *Pgt* is favoured by high humidity and warmer temperatures ranging from 15° to 40°C (Leonard and Szabo, 2005), where hot days ranging from 25° to 30°C and mild nights between 15° and 20°C, are considered optimal environments (Roelfs, 1984). According to Roelfs *et al.* (1992) urediniospores germinate within 1 to 3 h at a range of temperatures when in contact with free moisture, however a moisture or dew period of 6 to 8 h at favourable temperatures is necessary for germination. Successful entry into the host depends on the development of specialized structures of the germ tube. These infection structures, e.g. the appressorium, infection peg, substomatal vesicle and infection hypha, are characteristic for each rust species (Littlefield and Heath, 1979). Yirgou and Caldwell (1968) proved that in the absence of light, growth of *Pgt* stops after the formation of the appressorium and development will only resume as soon as the concentration of CO_2 is reduced. From the base of the appressorium a penetration peg grows through the stoma to produces an elongated substomatal vesicle in the substomatal cavity. Usually infection hyphae emerge from each end of the vesicle, growing intracellularly until they connects with a host cell where they differentiates to generate a haustorial mother cell, separated by a septum from the hypha (Leonard and Szabo, 2005). The haustorium mother cell produces a narrow penetration peg, which according to Harder and Chong (1984), use enzymatic dissolution as well as pressure in the penetration process to form a haustorium inside the host cell to extract nutrients from the host. The primary hypha develops into a branched, multicellular net of mycelium, producing urediniospores which eventually erupt through the surface. The infection process of *Pgt* is a complicated signalling and response system between host and pathogen. The penetration process is irreversibly stopped if moisture evaporates during the germination period (Roelfs *et al.*, 1992), therefore the availability of moisture is essential for the establishment of disease.

Rust spore dispersal is classified into short (within a crop), medium (between crops within a region) and long (from one region to another) distance transport. Wind is

responsible for rapid and extensive spreading of urediniospore inoculum (Eversmeyer and Kramer, 2000) such as the example of *Pgt* dispersal from Southern Africa to Australia (Brown and Hovmøller, 2002, Visser *et al*., 2019). Despite strict phytosanitary regulations the presence of urediniopores on travellers' clothes or on infected material increases the risk of intercontinental transfer of *Pgt* (Wellings, 2011). Therefore, physical barriers cannot prevent spreading of urediniospores to new hosts or regions.

Host range

Pgt has a wide host range with the ability to attack a variety of grass species in addition to the cultivated cereals. The host range also tends to be broader in regions near the centers of origin, such as the Transcaucasus, Middle-Eastern and Western Mediterranean countries where co-evolution of the host and pathogen has occurred, than in areas such as North America where it was introduced. The primary hosts for *Pgt* are species of *Aegilops, Elymus, Hordeum* and *Triticum*, producing urediniospores, teliospores, and basidiospores, whereas common barberry, several other *Berberis* and related species of *Mahonia* and *Mahoberberis* in the family Berberidaceae serve as altenate hosts for *Pgt* in the life cycle component that produces pycniospores and aeciospores. The most important alternate host is common barberry (*Berberis vulgaris* L.) which was introduced to the United States by European settlers. Eradication thereof started as long ago as in the mid-1600s as a means to control *Pgt* and has been practiced by many countries (Jin *et al*., 2010). The role of barberry in generating new virulence combinations was not known until heterothallism and the function of the pycnia as sexual organs of *Pgt* were discovered by Craigie (1927). Currently the sexual reproduction cycle has not been detected in SA on barberry and only two of 18 species cultivated are known to be susceptible to rusts (Keet *et al*., 2014). However *Bromus catharticus* Vahl (=*B. uniloides* H.B.K., *B. wildenowii* Kunth) an annual perennial (Kloppers *et al*., 1993) and wild (mouse, false or wall) barley (*Hordeum murinum* sensu lato) (Le Roux and Rijkenberg, 1987) are widely distributed and serves with volunteer or selfsown wheat as non-crop hosts. These are found growing in unplanted fields, on the edge of fields and roads or as weeds in the subsequent crop providing initial inoculum as ancillary host for wheat stem rust.

Triticale is a host of *Pgt*, but is hardly ever affected in the absence thereof on wheat during the cropping season. However out of season, it can be an important carrier of inoculum.

Life cycle

According to Voegele, Hahn and Mendgen (2009) De Bary (1853) was the first to note the importance of the germpore in urediniospore walls for germtube production. He also described the importance of tip growth direction for penetration through the stomata. Furthermore, in 1863, he introduced the term haustoria and, in 1865 elucidated the complex rust life cycle and defined the terms autoecious (completing their entire life cycle on a single host) and heteroecious (requiring two host species to complete their life cycle). Rust fungi are obligate biotrophs and therefore need a living host to reproduce and complete their life cycle (Fig 1.3). *Pgt,* with five distinct spore stages (macrocyclic), is heteroecious with wheat serving as main, economic host and common barberry as the alternate host.

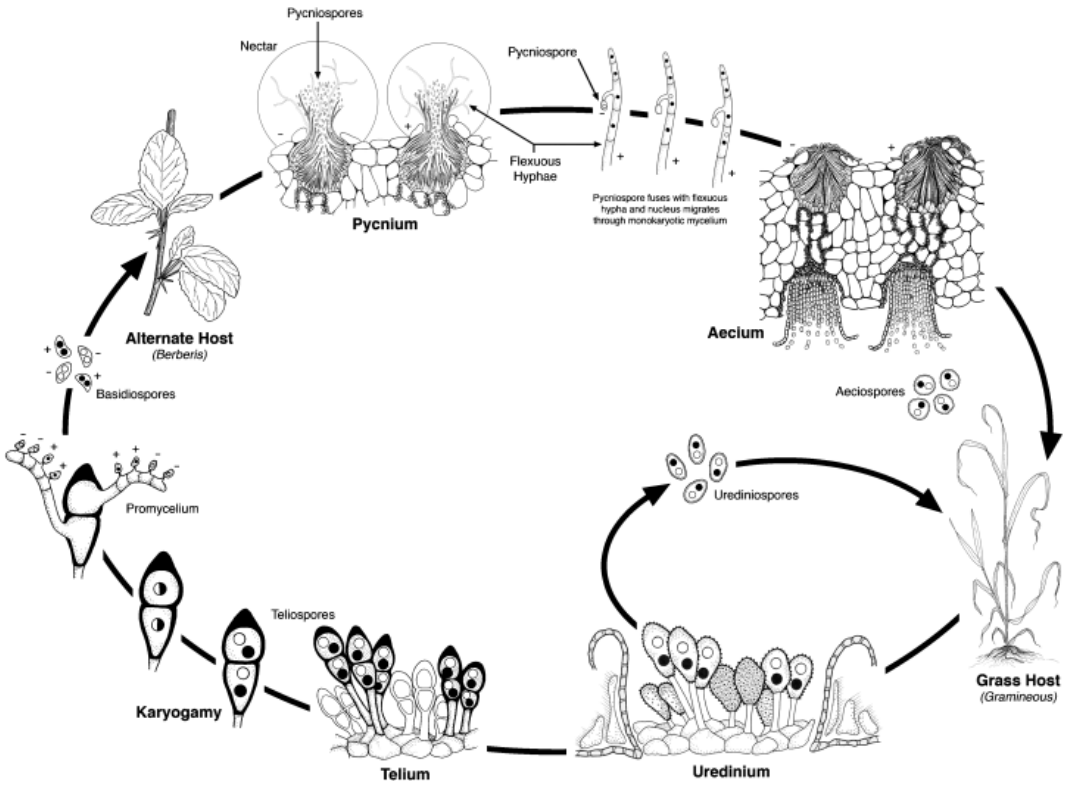

Figure 1.3: Schematic illustration of the life cycle of *Puccinia graminis* f. sp. *tritici.* (Leonard and Szabo, 2005).

The asexual phase of the cycle begins in wheat during cultivation with the germination of an urediniospore to form a germtube and penetration of wheat tissue by entering through the stomatal opening to develop intercellular mycelia with intracellular haustoria (specialized structures which absorb nutrients from the plant cell (Voegele and Mendgen, 2011). After establishing, the uredinia produce new elliptical, dikaryotic ($n^{+}n$) urediniospores with a thick-wall that is covered with spines (Harder, 1984) (Fig 1.4 A). These urediniospores are dehiscent and released as powdery masses and can easily be transmitted long distances by wind to re-infect not only already infected but also healthy wheat (Visser *et al*., 2019), (Fig1.4 B). As infected plants mature or when wheat plants die at the end of the season and temperature changes urediniospores cannot survive to infect a subsequent crop and therefore, to survive, formation ceases and shiny-black teliospore are formed, turning the infections black. These teliospores are thick-walled (with up to five wall layers), but two-celled and thickened at the apical end (Fig 1.4 C) and occur in the same lesion as the uredinial stage or in new (telia) fruiting structures. However, teliospores are sessile and therefore the spores are firmer and not easily released, but because they are dormant, they enable the fungus to survive on wheat debris (Mendgen, 1984). Mature teliospores are the only true diploid spore stage of the fungus.

As soon as weather conditions are favourable *Pgt* goes into the sexual stage of the life cycle where teliospores germinate and subsequent meiosis produces four haploid basidiospores, consisting of two of each opposite mating types, from each basidium. Basidiospores are thin-walled, colourless and easily blown away by wind to infect the alternate host by direct penetration through the epidermal cells, growing intercellularly to form haploid mycelium resulting in the formation of a spermagonium, a fruiting structure also known as a pycnium. The pycnia normally develop in clusters on the adaxial side of the leaf where they emerge as light chlorotic areas on young leaves, then change to light-orange-brown lesions resulting in small cone shape eruptions. The key feature of pycnia is the production of receptive hyphae and haploid spermatia (a.k.a. pycniospores) in a sticky and sweet nectar to attract insects which serve as transport to other flexuous hyphae of opposite mating types for fusion.

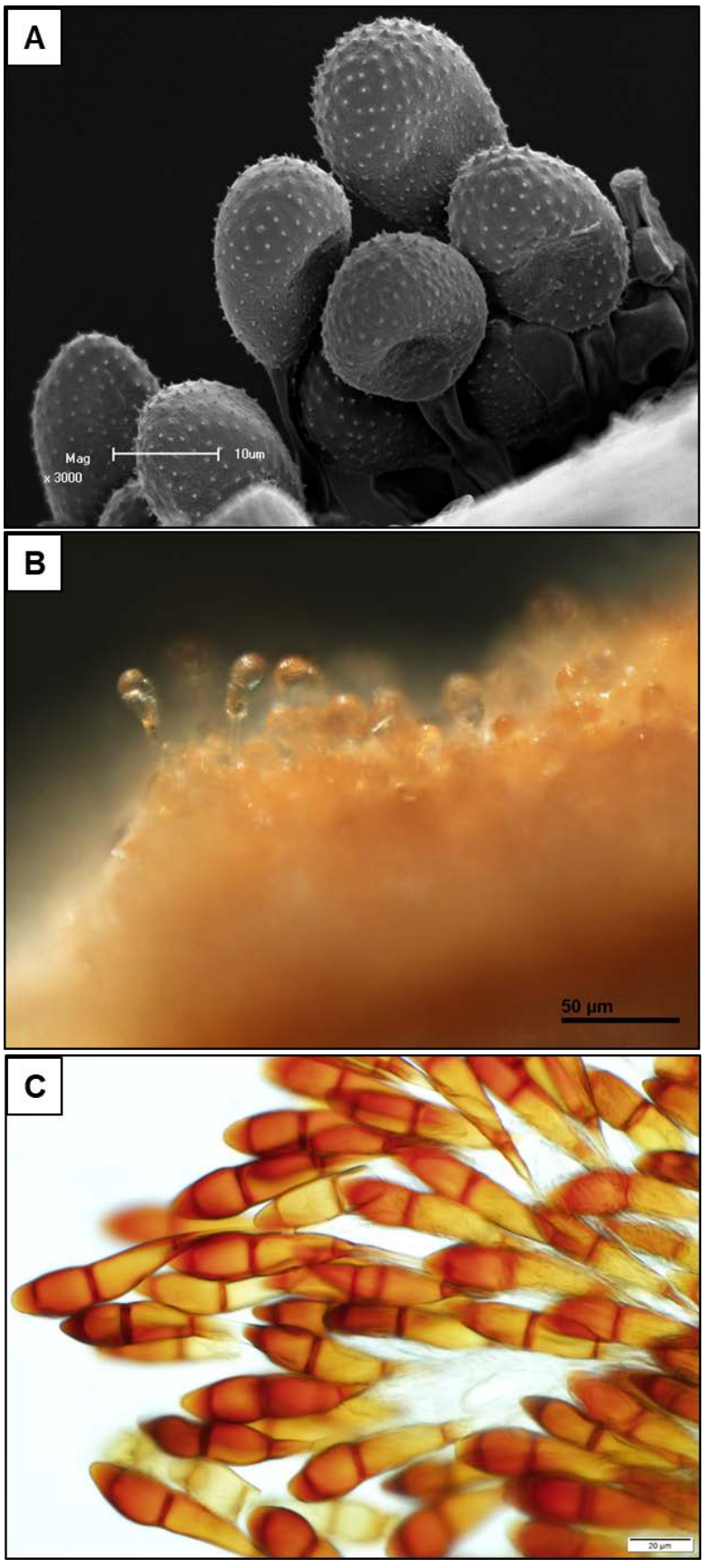

Figure 1.4: Urediniospores (A) and (B) and teliospores (C) of *Puccinia graminis* f. sp. *tritici*.

Spermatia are not able to infect plants, their only purpose is sexual reproduction which aids *Pgt* in producing new races. Rain splashing can also transmit the spermatia and after fusion, dikaryotization occurs to produce another fruiting structure, the aecium, usually on the lower side (abaxial) of the barberry leaf. Each individual aecium is cylindrical and flares out at their apices, but when closely packed in rings, the clusters of aecia appear bright orange when they mature (Fig 1.5). Dikaryotic aeciospores develop sequentially in chains from aeciosporophores to infect the wheat host through stomata and complete the sexual phase of the life cycle. In the absence of common barberry, asexual reproduction is enough to continue the life cycle by surviving cold temperatures as urediniospores on residues or volunteers of wheat.

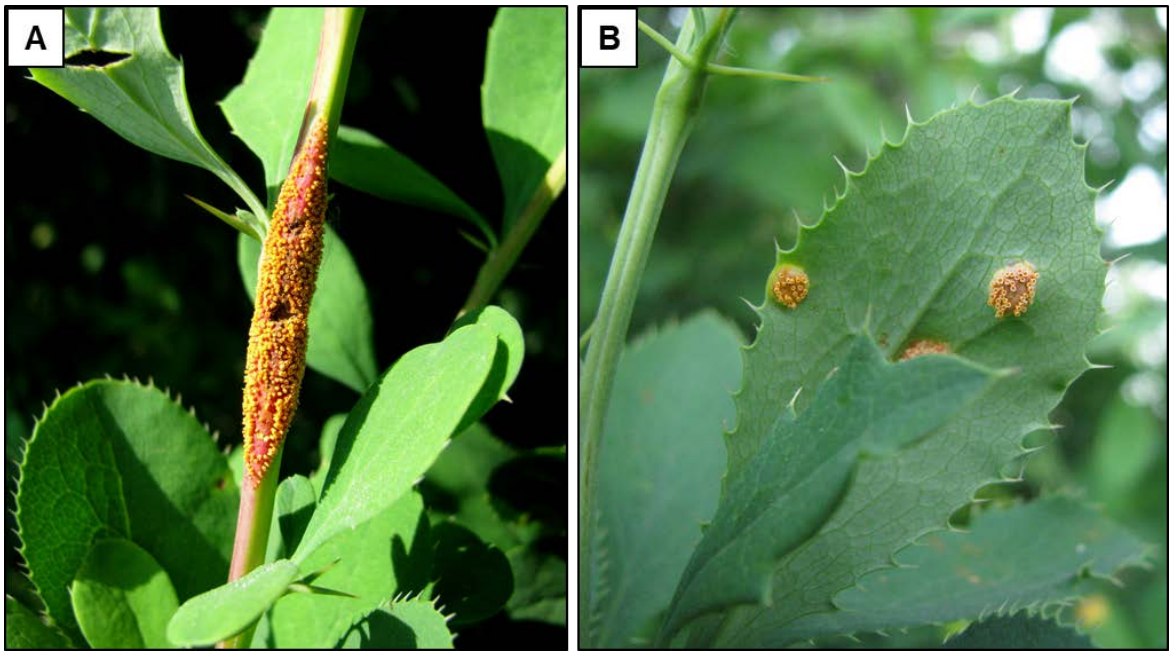

Figure 1.5: Aecia of *Puccinia graminis* on common barberry (A) stem and (B) abaxial side of leaf.

Nomenclature

Julich (1981) grouped rust fungi with smut fungi in the class *Teliomycetes*, but Cummins and Hiratsuka (2003) separated the phylum *Basidiomycota* into three classes namely *Urediniomycetes* (incuding the rust fungi), *Ustilaginomycetes* and *Hymenomycetes*. According to Maier *et al.* (2003) the *Uredinales* include more than 100 genera and around 7000 species. Currently the order is known as Pucciniales (accessed November 2019 https://www.cabi.org/isc/datasheet/45797 #totaxonomicTree)

Taxonomic tree

Domain: Eukaryota
Kingdom: Fungi
Phylum: Basidiomycota
Subphylum: Pucciniomycotina
Class: Pucciniomycetes
Order: Pucciniales
Family: Pucciniaceae
Genus: *Puccinia*
Species: *graminis*

Physiologic races

Most rusts, including *Puccinia graminis* which can infect up to 365 species of grasses and cereals, affect several hosts (Anikster, 1984). These rust species are divided into speciailized classifications, each labeled a *forma specialis* (f. sp. variety, specialized form) as determined by the host species and were originally described by Eriksson and Henning in the 1890's (Eriksson, 1894, Eriksson and Henning, 1896, in Voegele *et al.*, 2009). Within *formae speciales* rust races/pathotypes are assigned according to their virulence/avirulence pattern and the susceptibility/resistance pattern of their relevant host in line with the gene for gene hypothesis described by Flor on flax rust in 1955. However, different races were only identified in 1917 by Stakman and Piemeisal. In 1922 Stakman and Levine developed the first standard set of differential varieties (consisted of 12 varieties) to identify *Pgt* races within field collections. The updated set (Stakman *et*

al., 1962) has been used globally and still provides a taxonomic base for some race classification systems and therefore a means of comparison. These differentials include lines with diverse complexes of resistance genes making it difficult to define virulences of rust populations, because they differ within ecological zones or between different regions of the world. Thereafter numerous differential sets have been used and supplemented, which lead to a lack of reliability in race analysis between rust laboratories (Park *et al.*, 2011). This made comparison very difficult and a simplified international set of differentials (North American Stem Rust Nomenclature System) was accepted to ensure global consistency (Roelfs and Martens, 1988, Jin *et al*., 2008). Collaboration between wheat farmers, breeders and pathologists to monitor *Pgt* across the globe is done by utilizing trap nurseries or by pathotype surveys. The BGRI, the Rust SPORE web portal founded by the FAO and interactive applications like RustMapper and RustTracker, developed by CIMMYT are systems assisting to spread information worldwide (Park *et al.*, 2011).

Although the eradication of barberry and the improvement of resistance in varieties reduced epidemics, the 1953 and 1954 epidemics in the USA resulted from the appearance of a new race virulent to *Sr11* in a cultivar such as Lee. These epidemics furthermore affected several Hope (*Sr2*) and Thatcher derivatives, but despite this, they were later used as important and sustainable sources of resistance (Singh, 2015).

In South Africa the first pathotype surveys started in the 1920s, but were stopped until *Pgt* research commenced in the 1960s (Verwoerd, 1937, De Jager, 1980). Thereafter, regular surveys and the use of improved differential sets showed that rust epidemics were caused by different pathotypes and 18 were identified from 1920 to 1973. The host plants included *Agropyron distichum, Bromus maximus, Dactylis glomerata, Hordeum murinum*, *H. vulgare*, *Lolium italicum* and *Triticum aestivum* (Pretorius *et al.*, 2007). Since 1980 annual *Pgt* surveys have been conducted by the Agricultural Research Council (ARC) indicating that *Pgt* has developed virulence to various resistance genes, including triticale genes such as *Sr27* (Smith and Le Roux, 1992). The widespread use of varieties with single gene resistance most likely led to excessive selection pressure for changes in virulence

(Pretorius *et al*., 2007). Currently there are two major classification systems used in South Africa for wheat rust pathogens: the ARC and the University of the Free State (UV) systems. In the ARC system, the digit “2” indicates a stem rust pathogen therefore, a 2SAnumber would indicate stem rust sample number with a specific virulence/avirulence pattern, whereas the UV system uses “Pgt”number to designate a specific pathotype. These systems however have no biological or mathematical basis and only identify isolates with unique pathogenicity and cannot be used to trace individual virulence or avirulence characteristics.

The importance of races in wheat stem rust was re-emphasized by the detection of African race “Ug99” (North American race code TTKSK) in Uganda (Pretorius *et al.,* 2000). This discovery, in particular the occurrence of virulence for the commonly used *Sr31* resistance gene, has since initiated a global effort to combat the disease (McIntosh and Pretorius, 2011). At present 13 variants within the Ug99 race group are known, differing in virulence for *Sr9h*, *Sr21*, *Sr24*, *Sr30*, *Sr31, Sr36* and *SrTmp* (Singh *et al.,* 2011, 2015, Pretorius *et al.,* 2012, Patpour *et al*., 2015, Fetch *et al.*, 2016). Distribution of Ug99 variants has been confirmed in Egypt, Eritrea, Ethiopia, Iran, Kenya, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Uganda, Yemen, and Zimbabwe (Singh *et al.*, 2008, Nazari *et al.*, 2009, Visser *et al.*, 2011, Pretorius *et al.*, 2012, Newcomb *et al*., 2016, Terefe *et al*., 2019, www.rusttracker.cimmyt. org [accessed November 12, 2019]). More than 90% of all wheat cultivars and breeding material are rendered susceptible by this unique combination of virulences.

In South Africa, *Sr31* was not commonly used in varieties due to the linked secalin gene (codes for sticky dough), therefore Ug99 was not considered an immediate threat (Pretorius *et al.*, 2007). However when Visser *et al.* (2009) tested the South African pathotypes he noted that UVPgt55 (2SA88, TTKSF) is almost identical to Ug99, the only difference being the absence of virulence for *Sr31* (Pretorius *et al*., 2007). *Sr31* virulence was found in November 2009 on a wheat genotype suspected to carry *Sr31* in a rust nursery in KwaZulu Natal near Greytown. This isolate was also virulent for *Sr24* making it a considerable threat in South Africa. According to

Pretorius *et al.* (2010) it emphasizes the importance of avoiding single gene rust resistance in varieties as well as the continuous threat of pathogenic adaptation.

Ongoing non-Ug99 race associated epidemics cause major losses worldwide. In Southern Ethiopia, four stem rust races, TKTTF, TTKSK, RRTTF and JRCQC were associated with the epidemic caused primarily by TKTTF on the cultivar Digalu in 2013 (Olivera *et al*., 2015). In a rare stem rust outbreak in Germany in 2013, six races, TKTTF, TKKTF, TKPTF, TKKTP, PKPTF and MMMTF, were documented with race TKKTP indicating a unique virulence combination for *Sr24*, *SrTmp* and *Sr1RS^{Amigo}* (Olivera *et al*., 2017). According to Olivera *et al*. (2017) the German TKTTF isolate was phenotypically different from the Ethiopian isolate. In 2016 several thousand hectares of durum wheat on the Italian mainland and the island of Sicily were infected with stem rust races TTTTF and TTRTF, resulting in the largest stem rust epidemic in Europe in decades. Furthermore, the race TKTTF was identified in Sardinia (Patpour *et al*., 2017.) Although these races are not connected to the Ug99 group, it is important because of the combination of virulence for genes *Sr9e* and *Sr13,* both sources of resistance in many durum varieties. Without proper control these races could spread over long distances along the Mediterranean basin and the Adriatic coast (accessed November 2019 Rusttracker.cimmyt.org). Lewis *et al*. (2018) reported stem rust on wheat in the United Kingdom (UK) after an absence of nearly 60 years and documented for the first time the sexual stage on barberry and with only 20% of the UK varieties resistant to this race (TKTTF) it could result in major epidemics. Other challenging virulence combinations are *Sr22+Sr24* and *Sr13b+Sr35+Sr37* detected in Georgia from 2013 to 2015 (Olivera *et al*., 2019), where sexual recombination was acknowledged for the higher levels of phenotypic and genotypic ranges. The wide distribution of common barberry signposts Georgia as a hotspot for generating new virulence combinations.

DISEASE MANAGEMENT AND CONTROL

Stem rust can be controlled by different methods, however none of these is adequate on its own. As early as 1000 B.C. attempts to control stem rust involved religious practices one of which was, in the early 1600s, dragging of a rope over the grain to reduce dew deposition on plants (Worlidge in Roelfs, 1985). In 1660

laws required barberry eradication in France, but a successful barberry eradication program was only started in the USA in 1918 with E.C. Stakman at its head (Roelfs, 1982). In the absence of alternate hosts, the onset of rust is delayed because the establishment of disease now depends on wind-blown urediniospores and the initial inoculum is reduced. Furthermore, by excluding sexual recombination the risk of new pathogenic races decreases (Knott, 1989). Therefore, destroying alternate hosts interrupts the life cycle of rust fungi, which limits their diversity and although the battle has been effective in reducing pools of wheat stem rust it has not controlled the diseases. Currently stem rust is controlled primarily by either the use of chemicals or genetic resistance, and to a lesser extent by cultural methods (Knott, 1989).

Chemical control

Fungicides have been successfully used worldwide to control rusts, facilitating high yields and adequate wheat prices. Chemicals have a vast potential impact on the environment (Roelfs *et al.*, 1992, Bux *et al.*, 2012) and are in developing countries not always easily accessible or affordable. Usually biological fungicides are not as effective as chemical fungicides because multiple applications are required that need to be precisely planned to prevent disease, especially when environmental conditions favour disease development. A risk of chemicals is the likelihood that pathogens may develop resistance to fungicides, rendering them ineffective (Oliver and Hewitt, 2014). However, fungicides are an important part of the control strategy of stem rust, particularly when resistant varieties are not available or when an existing resistant cultivar becomes susceptible due to the emergence of a new race. In South Africa rust on wheat is generally chemically controlled with one to three applications in a single production season (Boshoff *et al.*, 2002), which is costly and an environment unfriendly approach.

Cultural practices

Cultural control is the practice of altering the growing environment of wheat, aiming at breaking the life cycle of *Pgt* at critical stages such as overwintering or oversummering. According to Roelfs (1985) most of the environmental conditions that favour wheat also favour rust. The importance of a green bridge (carrying the

diseases from one crop to the next) was emphasized by Zadoks and Bouwman (1985) particularly in the USA where the green bridge is extended when early planting may increase the chance for infection during autumn and successive overwintering of *Pgt* in milder climates or when some farmers plant early and others late. Effective practices include changing planting dates, use of early maturing varieties, multi-lines or varietal mixtures and destroying of volunteer plants or alternate hosts. Implementing only some or all of these measures does not guarantee the absence of disease, but may add to the success in controlling the disease when used in combination with resistance genes and fungicides.

Resistance breeding

The most effective way to control stem rust is by growing resistant varieties which reduces the use of fungicides, providing an environmental friendly and more cost-effective method of control. According to Browder (1985) resistance can be classified as any genetically determined characteristic of a host plant that in any way limits the damage caused by disease. Durable resistance defined by Johnson (1979) as "resistance that has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or period of time, in an environment favourable to a disease or pest" becomes even more important especially with constantly changing rust pathogens (Singh *et al.*, 2011). Furthermore effective gene stewardship, described by Pretorius *et al*. (2017) as “careful and responsible management of resistance genes” can help to protect resistance genes from the changing *Pgt* population. However to be efficient the entire breeding community needs to develop and deploy varieties that carry multiple resistance genes in various combinations and pathogen populations should be kept as small as possible to decrease the risk of mutation.

Stem rust control using resistant varieties has to deal with limited durability, because for years breeding programs worldwide were based on the deployment of a few single resistance genes. These genes are usually race specific and function only when the infecting *Pgt* pathotype lacks virulence. Therefore, these genes became more vulnerable due to selection pressure. Major race specific resistance genes are often defeated by the continual appearance of new *Pgt* races such as

the highly virulent stem rust race Ug99 (TTKSK) which adapted, spread and currently renders over 90% of the worlds' wheat varieties susceptible (accessed November 12, 2019 www.rusttracker.cimmyt. org). Prior to the appearance thereof in 1999, *Sr31* was effective for more than 30 years against all stem rust races worldwide and is used widely in breeding material (Pretorius *et al.*, 2000).

In genetic resistance there are commonly two groups of genes based on their phenotypic expression. The first group is pathogen race- or strain-specific resistance (ASR) genes which generally are expressed from seedling to adult growth stages, while genes in the second group functions primarily at the adult plant stage, therefore called adult plant resistance (APR) (Ellis *et al.*, 2014). When ASR genes are used singly they are considered non-durable, but when managed correctly they should contribute successfully to the control of stem rust. Lasting success lies in the use of varieties carrying several genes (gene pyramids or stacks) rendering loss of resistance rare. APR genes are characterized by less and slower rust growth, usually without necrotic responses, also referred to as partial or slow rusting (Ellis *et al.*, 2014). An example of a valuable race non-specific APR gene is *Sr2* which was transferred from wild emmer wheat in the 1920's (Mago *et al*., 2011). Although *Sr2* has been used globally in several breeding programs as the base for building rust resistance in new lines, *Pgt* has not overcome this gene. APR is best expressed in the field and selection thereof depends on optimized infections or epidemics. However, effective selection of APR genes in the field can be challenging because of masking of APR genes by ASR genes, especially those with strong resistance phenotypes. The use of specific races of *Pgt* to induce epidemics can assist to select APR genes. Although the use of APR genes is more complex than using ASR genes Singh *et al.* (2014) reported that the combination of several APR genes can result in "near immunity". It is however still essential to use the correct combination of resistance genes, because some combinations will not yield higher resistance compared to the individual effects. When combining genes using different resistance mechanisms or have varying optimal temperatures, better resistance can possibly be achieved in terms of protection under a wider spectrum of conditions (Lagudah, 2011).

Wheat has limited potential as a source for new or unused resistance genes (Martens and Dyck, 1989), whereas there is a reservoir of resistance genes among the relatives of cereals (Gerechter-Amitai and Loegering, 1977, Rahmatov *et al*., 2016b) especially a gene such as *Sr59* recently described by Rahmatov *et al.* (2016a) from rye possessing resistance against the widely virulent Ug99 (TTKSK).

The first interspecies crosses in SA were made in 1912 when emmer wheat was used in an effort to transfer rust resistance genes to wheat (Pakendorf, 1977). Since then wheat breeders and producers have tried to stay ahead by using rust resistance genes from *Agropyron elongatum* and *T. timopheevi*, eg. H44-24 and others, but these became susceptible in classic boom and bust cycles. Breeders up to the 1960s, unaware of the genetic variability of *Pgt*, bred varieties adapted to the different environmental zones of South Africa, which subsequently failed due to the absence of rust resistance. The cultivar Hoopvol remained in production from 1948 to 1971 probably because of horizontal resistance (Pretorius *et al*., 2007). Lately wheat breeding programs in South Africa have focussed more on adaptation (specifically drought resistance), quality and yield, than on disease resistance.

Traditional breeding approaches for gene transfer include backcrossing, pedigree or bulk selection, single seed descent and doubled haploid breeding (Allard, 1999) of which the fastest method to obtain a homozygous population is doubled haploidy (Ahmad *et al.*, 2016). These methods can be used on their own or in various combinations. However the success thereof depends on knowledge of the genetics of the donor and recipient parents, whether the resistance gene(s) can be expressed with no adverse effects on other traits for example yield or quality. These breeding methods are often time consuming, demanding extensive efforts such as multiple backcrossing, whereas modern breeding approaches in phenotyping, genetic modification and molecular markers accelerate and improve the precision of gene transfer or stacking. According to Pretorius *et al.* (2017) molecular genetics was established when DNA technologies were developed in the late 1980s to early 1990s and currently provide cut-edge tools to understand the genetics of rusts (Visser *et al.*, 2011) and the interaction between host and pathogen at cellular level. Advanced semi-automated (SSRs) and high-throughput applications (DArTs and

SNPs) superseded preceding technologies (AFLPs, CAPs, RAPDs, RFLPs, SCARs and STS markers) leading to enhanced efficiency in rust resistance breeding (Pretorius *et al.*, 2017). Globally the use of automated phenotyping by robotics and sensor imaging for data acquisition is expanding, enabling faster and more accurate identification of targeted traits. Mondal *et al.* (2016) stated that these new developments in remote sensing and high throughput technologies enable characterization of a large number of germplasm more rapidly, but high operational cost limit their large scale use in breeding programs.

Assessment of rust resistance

Accurate assessment of resistance is essential for the success of breeding for stem rust resistance and depends on an appropriate method for selection to develop new varieties carrying resistance genes (Ayliffe *et al*., 2013). Screening under field conditions is according to Niks *et al.* (1993) representative of commercially grown crops, but it is time consuming, expensive and the expression of resistance genes is dependent on different biotic and abiotic stress factors (Hickey *et al.*, 2012). In greenhouses the environmental conditions are highly controlled but less representative of actual growing conditions. However, inoculum type, quantity and uniformity of distribution can be manipulated to screen plants independent of growing field seasons (Niks *et al*., 1993).

Screenings of adult plants are done by assessing disease severity on stems based on the modified Cobb scale together with a host reaction type viz. resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S) (Peterson *et al.*, 1948). These ratings can include the whole plant or a designated area on the stem (stem leaf sheath and true stem). Under controlled environment conditions an infection type scale for stem rust, as described by Roelfs (1988), can be used on flag leaves. Seedling infection types range from 0 to 4 where 0 to 2 are usually considered to indicate resistance and 3 to 4 susceptibility.

Without quantification of stem rust, assessment of crop losses, studies on epidemiology and *Pgt* pathotype surveys and applications thereof, would not be possible.

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Chapter 2: Long-term assessment of South African wheat germplasm for stem rust resistance

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most consumed cereal crop in South Africa (SA) and is produced in both the summer and the winter rainfall regions with about 80% of the hectares planted under dryland. A consistent decline of hectares planted to wheat (Fig 2.1) is due to a decrease in profitability of the crop, making SA a net importer of half of the amount consumed annually (accessed November 2019 www.grainsa.co.za/2017/2018s-wheat-market-trends). Contributing factors to the significant decline in the area planted to winter wheat in the Free State Province are unreliable spring rainfall and higher winter temperatures resulting in severe moisture stress, especially on lower potential soils. Due to higher yield potential, between 30% and 40% of the total wheat harvest is produced under irrigation. Wheat cultivars are classified according to their vernalisation requirement as spring, intermediate or winter types.

Stem rust of wheat caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is commonly found in SA (Fig 2.2) (Terefe *et al*., 2016) with recorded yield losses of over 35% (Pretorius *et al*., 2007). In a recent study, Soko *et al*. (2018) documented a 47.9% decrease in yield in a stem rust susceptible wheat line whereas losses reached 19.5% and 6.4% in lines with adult plant resistance (APR) and all stage resistance (ASR), respectively. While chemicals can be used to manage stem rust, genetic resistance is accepted worldwide as the most effective approach. Pretorius *et al*. (2007) mentioned that rust control in SA has focused on resistance breeding due to the high costs and possible detrimental environmental effects of chemicals. Despite this strategy, most if not all wheat producers in the Western Cape currently follow a rigorous chemical application programme aimed at foliar disease control.

Thirty two *Pgt* pathotypes have been identified in SA through surveys since the early 1980s (Terefe *et al*., 2016, Boshoff *et al.*, 2018b, Terefe *et al.*, 2019.) This variability and regular occurrence of new pathotypes emphasize the importance of resistant germplasm and therefore the annual screening of all listed SA wheat

varieties as well as advanced breeding lines. The Agricultural Research Council-Small Grain (ARC-SG), and the commercial breeding companies Pannar and Sensako, contribute varieties and lines for rust screening on an annual basis. Previously these breeding establishments requested the objective assessment of wheat varieties for rust response by an impartial organization not actively contending in the seed market. The South African Winter Cereal Trust has financially supported the annual assessment of rust response for provisionally and finally released wheat breeding lines since 2007. The assays include seedling screening in the greenhouse at the University of the Free State, Bloemfontein and field trials at PANNAR Research Station, Greytown, KwaZulu-Natal and on occasion at Makhathini Research Station 20 km north-east of Jozini on the Makhathini flats, KwaZulu-Natal. Each year the cultivar recommendations for rust response are published by the ARC-SG in the “Guidelines for Production of Small Grains in the Summer Rainfall Region” and “Guidelines for Production of Small Grains in the Winter Rainfall Region”. These risk assessments assist growers in cultivar selection and disease management based on reliable long-term data, which are revised annually to make provision for new, improved cultivars as well as for the influence of pathotype changes.

The Western Cape is currently considered the breadbasket of SA (Fig 2.3) and contributes almost 50% of the production (www.grainsa.co.za/pages/industry-reports/production-reports) (South African Grain Laboratory Wheat Report 2018/2019). It is a winter rainfall region where spring wheat is planted in late autumn. Winter rain should preferably fall from May to September, whereafter the presence of moisture increases the possibility of diseases. *Pgt* prefers higher temperatures, therefore mostly appears later in the season when the plant is in the grain filling stage. In 2017 a significant level of *Pgt* infection only occurred after the soft dough stage of crop development (Terefe *et al*., 2017). Currently stem rust susceptible cultivars contribute to the higher incidence of the disease in the Western Cape where occasionally localized epidemics have occurred.

To ensure profitability for local wheat producers cultivar selection is a critical decision in input cost and risk management. To maximise yields the deployment

and production of resistant cultivars can help neutralize the risk of stem rust epidemics. Since stem rust occurs almost wherever wheat is grown, it has the potential to cause severe damage. An effective strategy for sustainable management of stem rust can be achieved through resistance breeding and the planting of well-characterised cultivars allowing optimised preventative control measures in high-risk areas. The main objective of this study was to characterise South African wheat varieties which include commercially available cultivars and advanced breeding lines, for seedling and adult plant stem rust resistance.

MATERIAL AND METHODS

Wheat genotypes

Using controlled inoculation procedures, wheat accessions obtained from all SA wheat breeding companies were characterised over nine years using either the most dominant or most virulent pathotypes of *Pgt* in the greenhouse and field. For this study wheat varieties included wheat cultivars and advanced breeding lines of hexaploid wheat. Annually the numbers and usually the entries varied according to the needs of each breeding company. However at least 26 wheat cultivars were included for 3 seasons from 2015 to 2017 of which 17 were assessed for 5 years from 2013 to 2017. The *Pgt* susceptible wheat cultivar SST 88 has been included in all trials since 2009. Susceptible checks, varying according to pathotypes, were included in all greenhouse seedling trials. The susceptible Line 37-07 served as a spreader and susceptible check in field trials. Line 37-07, pedigree: Kasyob/Genaro-81//Cham4, was selected from entry 37 of the 2nd International Stem Rust Trap Nursery (ISRTN07) based on its susceptibility to stem rust in field trials in South Africa.

Stem rust pathotypes

The seven *Pgt* pathotypes used for inoculation of SA wheat varieties, their ascribed codes [North American (NA) nomenclature (Roelfs and Martens, 1988, Fetch *et al.*, 2009), University of the Free State and SA codes] and their avirulence/virulence gene combinations as described by Terefe *et al*. (2016) and Boshoff *et al.* 2018b are shown in Table 2.1. These *Pgt* pathotypes were multiplied on susceptible wheat lines selected according to the resistance genes they carry (Table 2.1). For seedling

trials predominant pathotypes UVPgt55 (TTKSF), 57 (BPGSC+*Sr27*), 60 (PTKST) and UVPgt61 (TTKSF+*Sr9h)* were selected according to their incidence and importance in 2015 and 2016. Although considered relatively avirulent, UVPgt54 (BPGSC) and UVPgt62 (BFBSC+ *Sr27*) were added in certain years to assess their potential impact (Table 2.1). For field trials only one stem rust pathotype was selected according to its frequency across major wheat growing areas and / or pathogenicity (Terefe *et al*., 2016) and inoculated onto spreader rows to establish early infection and consequent epidemic development. Before the occurrence of UVPgt60 (*Sr24* + *Sr31* virulence) (Pretorius *et al*., 2010), pathotype UVPgt59 (*Sr24* virulence, *Sr31* avirulence) was used during 2009 and 2010.

Rust multiplication

Seed from susceptible wheat lines were planted in Mikskaar® potting substrate MPS2, for multiplication of urediniospores of selected *Pgt* pathotypes (Table 2.1). Where possible, wheat lines were selected to act as a selective host for each pathotype, thus confirming purity. Emerging wheat seedlings were drenched with 50 ml of a 99% maleic hydrazide ReagentPlus® [Sigma-Aldrich (0.3 g/L water) solution per 10 cm plastic pot to retard plant growth and enhance sporulation (Knott 1989). Greenhouse temperatures were set to an 18-25°C night/day schedule and plants were fertilized with water soluble Multifeed-Classic (Efekto®, NPK Analysis 19:8:16 (43), concentration 2.5 g/L water) twice a week. These procedures were performed for all wheat seedling evaluation trials except tha maleic hydrazide was not applied.

Urediniospores (stored at -80°C) were heat shocked at 46°C for 6 min before spray-inoculation of seven day old wheat seedlings of the respective multiplication hosts (approximately 1.5 mg spores /ml Soltrol® 130 (Chevron Phillips, Borger, Texas)). After inoculation wheat seedlings were dried in a growth cabinet at 25°C for about 1 h before incubated in darkness in a dew simulation chamber at 18-23°C and >96% relative humidity for 16 h. From the dew chamber the seedlings were returned to a growth cabinet fitted with fluorescent growth tubes (Eurolux G135, G13 9W T8 LED) for at least 2 h. Seedlings were then transferred and maintained under previously described conditions in isolation cabinets in the greenhouse until sporulation and collection of spores for inoculation of either seedlings or field trials.

Greenhouse evaluation of South African varieties at seedling growth stage

Five seeds from each accession were planted in 10 cm plastic pots with five accessions per pot. At the one and half leaf stage, approximately seven to nine days after planting, the seedlings were spray-inoculated with freshly harvested urediniospores of a single pathotype (concentration ~3 mg/ml), dried off, incubated in the dew chamber and returned to the greenhouse as described for multiplication of spores. Twelve to fourteen days after inoculation plants were scored according to the 0-4 seedling infection type (SIT) scale (Fig 2.4), where 0 = immune, ; = flecks, c = chlorotic, or n = necrotic, 1 = minute uredinia, 2 = small to medium sized uredinia, 3 = large uredinia sometimes encircled by slim chlorosis, 4 = large uredinia without any chlorosis, X = random spreading of variable-sized uredinia on a single leaf, Y = ordered spreading of different sized uredinia with larger uredinia at leaf tip and Z = ordered spreading of different sized uredinia with larger uredinia at leaf base (Roelfs *et al*., 1992). Infection types (ITs) of 3 or higher refer to a high IT (susceptible) while less than 2 were considered a low IT (resistant) (Table 2.2.1). A quality control score, out of 10 for each seedling experiment, was determined taking into account infection frequency and even distribution of pustules on leaves. Based on this quality control score, tests with certain pathotypes were repeated in a particular year, until a quality control score of >7.5 was obtained.

Field evaluation of South African varieties at adult plant growth stage

The wheat varieties were evaluated from 2009 for stem rust severity and reaction type in an annual rust nursery of the University of the Free State field trial at PANNAR Research Station, Greytown and in 2015 at Makhathini Research Station, KwaZulu-Natal, SA. Greytown trials were established two weeks apart each year with winter types planted late in May and spring types early in June. Plots consisted of a single 1 m row with an inter row spacing of 75 cm. A rust susceptible genotype (either Line 37-07, Morocco or McNair) was included at 10-row intervals and spreader rows (mostly a mixture of Line 37-07, Morocco and McNair) were grown orthogonally between blocks and as a trial border of at least two rows. Prior to planting, during seed bed preparation, 250 kg 2:3:4 (38) N-P-K plus 0.5% Zn was applied per hectare and the trials were irrigated to supplement rainfall and ensure favourable conditions for plant growth and rust development. Spreader rows were

spray inoculated with a concentrated inoculum suspension of urediniospores in Soltrol® 130 oil. Following inoculation, plants in certain row sections were allowed to dry off for approximately 1 h before covering them with plastic sheeting for 12 h. Before securing the plastic cover the soil around inoculated plants was watered to promote high humidity inside the plastic sheeting. This process was repeated two to five times per season to establish a homogeneous infection throughout the nursery. Makhathini trials were planted as hill plots during middle to late April and spray-inoculated with UV60 during the stem elongation to booting stages. Supplemental irrigation was applied as necessary.

Wheat host plant responses to *Pgt* were recorded according to the modified Cobb scale at the peak of stem rust development as severity (percentage stem area infected) (Peterson *et al*., 1948) and reaction type, based on the size of pustules and the amount of chlorosis, necrosis and sporulation (Roelfs *et al*., 1992). These include R = resistant, (no uredinia present); MR = moderately resistant, (small uredinia with necrosis and light sporulation); MRMS = moderately resistant to moderately susceptible, (small to medium-size uredinia with moderate sporulation); MS = moderately susceptible, (medium-size uredinia with moderate to intensive sporulation) and S = susceptible, (large uredinia with abundant sporulation) (Fig 2.5) (Roelfs *et al*., 1992).

Data analysis

Statistical analysis of stem rust data from field plots was not possible due to varying varieties numbers and entries over seasons. Owing to space and seed constraints, entries were grown in unreplicated 1-m rows per field season. To avoid inaccurate conclusions, a qualitative scale was used (Table 2.2.2) and the frequency in each group was quantified to assess stem rust resistance over years.

Coefficient of Infection

Disease severity and host reaction type are often combined into a single value, referred to as the coefficient of infection (CI), to compare different wheat varieties using a criterion that is more representative of the phenotype in the adult plant stage (Fig 2.6). The CI, where severity according to the modified Cobb scale is multiplied with a constant for host reaction type, where R = 0.2, RMR = 0.3, MR = 0.4, MRMS

= 0.6, MS = 0.8, MSS = 0.9 and S = 1, was calculated (Roelfs *et al*., 1992). In the present study varieties with CI values of 0 - 19 were regarded as resistant, 20 – 39 as intermediate and 40 -100 as moderately susceptible to susceptible (Table 2.2.2) (Pathan and Park, 2006).

RESULTS

The number of SA wheat accessions, evaluated over nine years, varied over years with a maximum of 145 evaluated for both seedling and adult plant response in 2015 (Table 2.5). In 2013, 192 wheat varieties were evaluated in the greenhouse for seedling resistance (Table 2.3) but due to drought conditions, no stem rust epidemic could be established in the field. In general, the standard of seedling data gathered in the greenhouse was considered high. A quality control score of 7.5 out of 10 was considered satisfactory but most seedling experiments received a score of 9 or 10.

Greenhouse evaluation of South African wheat varieties at seedling growth stage

SITs of SA wheat varieties varied from immune to susceptible within or between different pathotypes of *Pgt* and are presented in Tables 2.3 to 2.7. Some varieties showed both resistant and susceptible reactions indicated as mixed IT scores: e.g. 4p;1, 1p2 where 4 plants had a score of ;1 while a single plant had a score of 2. Less than 7.5% (2012) of entries expressed mixed reactions with the minimum only 1.7% (2014) and a mean of 3.6 % from 2013 to 2017.

Among the checks (last entries in Tables 2.3 to 2.7), Morocco and McNair 701 (*SrMcN*) were used as the universal susceptible controls (susceptible to all *Pgt* pathotypes tested). Barleta Benvenuto (*Sr8b*) showed susceptible SITs to pathotypes UVPgt55, 59, 60 and UVPgt61, whereas the SITs for pathotypes UVPgt57 and UVPgt62 were X (mesothetic) as reported by Singh and McIntosh (1986), indicating avirulence (Table 2.6). The triticales Satu (*SrSatu*) and Tobie (*Sr27, SrSatu*) were susceptible to pathotype UVPgt57, whereas Coorong (*Sr27*) was susceptible to both UVPgt57 and UVPgt62. Federation*4/Kavkaz (*Sr31*) and *Sr31* (Benno)/6*LMPG (*Sr31*) were susceptible to pathotype UVPgt60, whereas Matlabas (*Sr9h*) was resistant to UVPgt60, but susceptible to UVPgt61.

Since 2013 an eminent group of SA varieties expressed high SITs (susceptibility) to pathotypes UVPgt55, 60 and UVPgt61, varying between 34% to 57% of entries, except in 2015 when a lower percentage (26%) was susceptible to UVPgt55. In contrast, most entries were resistant to pathotypes UVPgt54, 57 and UVPgt62. (Table 2.8). Although the proportion of varieties, inoculated with UVPgt57, that showed a susceptible response increased from 12% in 2009 to 21% in 2010 (data not shown) and 17% in 2013 it sharply decreased thereafter (Table 2.8).

Resistance to UVPgt55, the most commonly occurring pathotype since its detection in 2000 (Boshoff *et al*., 2002, Terefe *et al*., 2016), increased from 48% (2013) to 73% (2015), but declined again to 54% in 2017 (Table 2.8). In comparison with UVPgt59, the number of entries with resistance declined rapidly following the introduction of the new pathotype UVPgt60. In 2009, 31.1% of wheat varieties indicated resistance as opposed to 11.9% in 2012 (Fig 2.7). Thereafter resistance improved to 33.8% in 2016, but decreased to 25.9% in 2017. However, in the latter year, more entries expressed intermediate resistance (Fig 2.7).

Field evaluation of South African wheat varieties at adult plant growth stage

The observed *Pgt* severities and reaction types of the SA wheat varieties for 2014 to 2017 are presented in Table 2.4 to Table 2.7. Grouped data, based on the CI of the wheat varieties tested from 2009 to 2017 (data from 2009 to 2012 not shown in tables) are presented in Figure 2.8. From these data it is clear that higher levels of *Pgt* resistance occurred against UVPgt59 in 2009 (64%) and 2010 (66%). However, in 2011 after the introduction of UVPgt60 only 34% of the varieties were resistant, improving to 44% in 2012, fluctuating in the fifties from 2014 to 2016 and restoring the higher levels of resistance (60%) in 2017.

A major decline in resistance, from 75% in 2010 to 7.8% in 2011, was observed in the cultivars planted in the Western Cape, but resistance gradually increased to 76% in 2017 (Fig 2.9).

Seedling and adult plant resistance comparison

Resistance to stem rust, broadly classified as major gene or ASR, can be detected in wheat seedlings and usually at all subsequent growth stages whereas APR is most effective in mature plants in the field (Lagudah, 2011, Ellis *et al*., 2014). Therefore, only SA varieties with SITs < 2 and CI < 20 were considered as carrying ASR. Some varieties with SITs <2 and therefore classified as resistant, grouped in the intermediate group as adult plants resulting in a slightly higher percentage of entries that expressed seedling resistance when compared to ASR (seedling resistance = ASR in definition) (Fig 2.10). The opposite was also observed where some varieties with SITs of 2 were classified as intermediate in the seedling group, but resistant as adult plants.

Inconsistent results were observed for some of the breeding lines included in the trials. Among the 2011 ARC-SGI winter elite entries, three lines were resistant as seedlings (ITs = ;11) but had field scores of 90MS and 90S (two lines). In 2012 one line with a SIT = 1 showed 40S in the field, in 2016 three lines with SITs = 11++ showed field scores of 70MS and 100MSS (two lines), and in 2017 two lines with IT = ;1 displayed field scores of 50MS and 70MS. Two of the spring elite lines from Pannar in 2015 with SITs = 1 showed higher field responses of 70MRMS and 70MS in adult plants. Evaluations of these breeding lines were not repeated over years, therefore possible varying results could not be excluded as was possible with cultivars that were evaluated for more than one year.

DISCUSSION

Currently the most common methods of stem rust control in SA are growing resistant cultivars and the application of fungicides. Since the release of the stem rust resistant cultivar Palmiet (carrying the APR gene *Sr2* and the ASR gene *Sr24*) in 1985, stem rust epidemics were reduced in SA pending the appearance of *Pgt* pathotype 2SA88 (UVPgt55) (Boshoff *et al*., 2002). Pretorius *et al*. (2007) mentioned that SA wheat cultivars did not have a broad-base *Pgt* resistance when pathotype UVPgt55 was first detected in 2000 where virulence for *Sr8b* and *Sr38* rendered about 80% of cultivars susceptible. This statement is supported by the results of Chemonges *et al.,* (2018) who reported that resistant intercrosses

between the SA winter wheat cultivars SST 387, Koonap, Komati and Limpopo yield no susceptible offspring to pathotype UVPgt60 indicating monogenic single gene resistance. Except for *Sr24* avirulence, UVPgt55 is, according to Visser *et al*. (2011), phenotypically identical to UVPgt59, which again is similar to UVPgt60 except that UVPgt60 has virulence for both *Sr24* and *Sr31*, but produces a slightly lower SIT on *Sr21*. Another pathotype with a similar virulence profile to 2SA88 is 2SA88+*Sr9h* (UVPgt61) with virulence towards the winter wheat cultivar Matlabas (Pretorius *et al.,* 2012b). Terefe *et al*. (2016) confirmed with simple sequence repeat (SSR) analysis the genetic relationship between these four pathotypes (UVPgt55, 59, 60 and UVPgt61) and Ug99. These *Pgt* pathotypes do not share a high genetic similarity with SA stem rust pathotypes detected before 2000. UVPgt55 is the most commonly found in all the major wheat producing areas whereas, UVPgt59 is found in the Western Cape and UVPgt60 mostly in the Free State and KwaZulu-Natal (Terefe *et al.,* 2016). According to Pretorius *et al*. (2010) regional SA *Pgt* epidemics followed the breakdown of genes such as *Sr9e* and *Sr24* in wheat and *Sr27* and *SrSatu* in triticale. Therefore, the decline from 2009 to 2012 in seedling resistance possibly followed the breakdown of genes such as *Sr9e, Sr24* and *Sr31* either alone or in combination. Although *Sr24* virulence had already been reported in 1987 (Le Roux and Rijkenberg, 1987) as well as *Sr8b* and *Sr38* in 2000 (Boshoff *et al.*, 2002), the combined increased virulence with *Sr31* in UVPgt60 caused a threat to wheat production in SA (Pretorius *et al.,* 2010). When Pretorius *et al*. (2012a) screened SA varieties for *Sr2*, *Sr24* and *Sr31*, 88% were susceptible as seedlings to at least one of the pathotypes tested. Recently the percentage of SA wheat varieties carrying *Sr24* was postulated to be between 18 and 23% (Pretorius *et al.,* 2012a, Terefe *et al.,* 2019). A feasible explanation for the frequent presence of *Sr24,* originally derived from *Agropyron elongatum* (Host) P.Beauv. (syn. *Thinopyrum ponticum*, http://www.globalrust.org/gene/sr24), in SA varieties might be its linkage to *Lr24* (Jin *et al*., 2008). The decrease of seedling resistance from 2009 to 2012 indicates the presence of some of these genes in certain SA varieties. However, the selection for resistance and the withdrawal of susceptible varieties in the following years resulted in an increase of genotypes with low to intermediate SITs.

The *Sr31* resistance gene originates from an introgression from the short arm of rye (*Secale cereale* L.) chromosome 1 (1RS) into wheat (1BS) (Singh *et al*., 2008). Wheat lines carrying this translocation were widely used in wheat breeding due to linked resistance to the rusts (*Sr31*, *Lr26*, *Yr9*) and wide adaption which resulted in more stable and higher yields (Ren *et al.,* 2012). The Ug99 group of *Pgt* races was recognized as a major threat to wheat production and food security capable of attacking 90% of world's wheat cultivars (Singh *et al.*, 2011). However, in SA with its strict wheat quality release criteria, cultivars carrying the 1BL-1Rs chromosome translocation are often turned down as it is associated with sticky dough creating problems in automated bakeries. Furthermore, the use of *Sr31* was discouraged in breeding in SA when virulence was documented in East Africa (Pretorius *et al*., 2000, 2010). The frequency of *Sr31* is therefore low in commercially available cultivars (Pretorius *et al*., 2012a).

Pgt pathotype UVPgt53 with virulence for *Sr27,* first detected in SA in 1988, rendered about 75% of commercial triticale cultivars susceptible (Smith and Le Roux, 1992). One of the predominant pathotypes surveyed in SA from 2009 to 2013 was UVPgt57 (virulence for *Sr27*, *SrKw* and *SrSatu*) (Terefe *et al*., 2016). It was observed for the first time in the Western Cape in 2005 (Roux *et al*., 2006) and probably developed through single step mutation from race UVPgt56 lacking comparatively only virulence for *SrSatu*. The combination of planting susceptible commercial wheat cultivars and triticale, as either a pasture or a cover crop, possibly add to the presence of UVPgt57 in the Western Cape. In 2010 UVPgt62 (virulent to *Sr27* and avirulent to *Sr9b*, *Sr11*, *SrKw* and *SrSatu*), related to the triticale pathotypes, was identified from Coorong samples (Terefe *et al*., 2016). SITs from 2014 to 2017 demonstrate that most SA wheat varieties are resistant to the triticale pathotypes UVPgt57 and UVPgt62 (virulence for all or one of *Sr27, SrKw* and *SrSatu*). The absence of virulence towards resistance genes such as *Sr5*, *9e, 24* and *Sr31* among the triticale pathotypes may explain their relative avirulence noted on wheat in this study. However, the same is not true for variants of the Ug99 group, such as UVPgt55, 59, 60 and UVPgt61 (Terefe *et al*., 2016).

Screening for resistance under field conditions is representative of commercially grown crops (Niks *et al*., 1993). Therefore, in this study the most virulent *Pgt* pathotype was used to establish infection in the field instead of using a mixture of pathotypes to allow for all the virulence combinations available. This was to prevent field dominance of a less virulent pathotype in a particular year through early establishment on the spreader rows. The only virulence not accounted for in the field data collected in this study is for *Sr9h* as the pathotype UVPgt61 is considered less virulent or important in a SA context than UVPgt60 with virulence for the widely deployed *Sr24* as well as for *Sr31* (Pretorius *et al*., 2010).

Comparative APR studies support the decline in resistance against pathotype UVPgt60 (*Sr31* virulence) in 2011. Progress made in the selection for resistance as well as the removal of susceptible SA cultivars and breeding lines led to an annual increase in overall APR since 2011. Resistance genes such as *Sr9e, 9h, 24, 31, 36* and *Sr38* were exploited as single genes by breeders in the development of commercial wheat cultivars in SA, therefore placing the stem rust resistance on a restricted genetic base (Le Roux and Rijkenberg, 1987, Boshoff *et al*., 2002, Wessels *et al*., 2019). Pretorius *et al*. (2008) emphasized that only a few SA cultivars have broad-based resistance protecting the genes involved. Single-gene resistance is often short-lived and prone to rapid breakdown with the emergence of a new pathotype. Results from this study support the use of multigene resistance, because the onset of the new pathotype UVPgt60 in 2010 (combined *Sr24* and *Sr31* virulence) led to a major decline in resistant varieties, especially in the Western Cape which accounts for more than 50% of SA wheat production. Previous studies showed that the incidence of stem rust was consistently higher in the Western Cape than in other wheat growing areas of SA (Boshoff *et al*., 2000, Komen, 2007, Terefe *et al*., 2010, Terefe *et al*., 2016). Although pathotype UVPg60 has been absent in the Western Cape (Terefe *et al*., 2016), it is possible that significant losses could be incurred once it spread to this spring wheat producing region. Probably due to routine application of fungicides the regular occurrence of stem rust in the Western Cape did not necessarily lead to noteworthy epidemics in recent years. At present *Pgt* pathotypes with virulence to *Sr24* constitute a threat to

wheat production especially in the Western Cape where susceptible cultivars are still commonly planted although resistant cultivars are available.

According to Pretorius *et al*. (2017) ASR (previously referred to as seedling resistance) genes provide rust resistance throughout all the different growth stages of the wheat plant. In this study wheat lines with SITs <2 did not consistently group in the resistant group when the seedling data is compared with the field data in Fig 2.10. This can be attributed to the high severities recorded in field epidemics in this study where the calculated CI values in some instances resulted in an intermediate classification despite strong RMR host reaction types. Despite the grouping of these entries in the intermediate group their host reaction types indicates that they do carry efficient levels of resistance, considering the lower inoculum levels often experienced in commercial fields. The specific gene(s) involved may also have an influence in host response grouping as the ASR genes *SrTmp* and *Sr21* have been reported as ineffective under conditions of severe stem rust pressure in the field when the *Pgt* pathotype UVPgt60 in fact is considered avirulent on seedlings (Boshoff *et al*., 2018a). Conversely, it was reported that wheat lines that carry the stem rust ASR genes *Sr9e, 9g, 17,* and *Sr30* do show APR and are not completely susceptible to pathotypes considered virulent on them in the seedling stage (Jin *et al*., 2007, Boshoff *et al*., 2018a). Rahmatov *et al*. (2015) developed double wheat-rye translocation lines with different translocations. One is the 1BL.1RS translocation derived from the rye cultivar Petkus that carries resistance genes against stem rust (*Sr31*), stripe rust (*Yr9*), leaf rust (*Lr26*) and powdery mildew (*Pm8*) (Zeller 1973, Friebe *et al*., 1996). When tested with varying virulent pathotypes, no major seedling resistance genes were confirmed in these lines, but APR was observed. According to these authors resistance was likely due to “several minor genes and the resistant reaction possibly based on additive or epistatic interactions”. Another possible reason for the discrepancy noted between seedling and ASR in this study could include segregation for resistance not noted in either seedling or field evaluations for specific entries. Verification of this was mostly not possible as the bulk of the entries were included only once.

With the sharp decline in wheat hectarage in SA over the last two decades, breeders are under pressure to counteract this trend through releasing higher yielding varieties. In general, the wheat potential of SA cultivars is higher than the yield currently achieved under commercial conditions. Explanations include environmental conditions, crop management decisions, and disease, insect pest and weed pressure (Anonymous, 2018). Despite some easing of the strict release criteria for milling and baking quality in recent years, this preoccupation with quality traits has often been blamed for a general lack of progress in breeding for higher yields. However, trends of rising winter temperatures and less reliable spring rain in especially the Free State Province, have resulted in a shift to summer crops considered as a lower risk farming practise.

Breeding for rust resistance is considered one of many traits that breeders need to consider. To achieve a more focused approach towards confirmation of target genes, a Marker Service Laboratory (MSL) was established by CenGen (Pty) Ltd. in 2011. Funded by the Winter Cereal Trust the MSL was set up to assist wheat breeders to progress with traits such as rust resistance, Fusarium head blight resistance and preharvest sprouting tolerance. From 2011 to 2018 the MSL generated more than 580 000 data points with a calculated 52% of those for rust resistance (R. Prins, personal communication). It is anticipated that this approach will result in the release of wheat cultivars with more complex and durable sources of rust resistance in the near future.

The results of this study include the most comprehensive characterization of SA wheat varieties over time for stem rust resistance. Producers have benefitted from the results through the annual update of cultivar responses in production guidelines. Data made available to breeders annually allowed them the opportunity to made adjustments in their breeding strategies for stem rust. Conventional wheat breeding involves 12-year breeding cycles and it is important that breeders anticipate potential virulence changes by the rust pathogens. A sound understanding of the basis of resistance in their germplasm and the continuous identification, incorporation and deployment of novel, more complex sources of resistance through applying the latest technologies, is required. The appearance of new

pathotypes, as illustrated with UVPgt60 in this study, can reverse progress made with resistance breeding when based on monogenic sources. The data generated has further increased our understanding of the different sources of stem rust resistance and their expression under both greenhouse and field conditions.

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Table 2.1: Avirulence/virulence combinations of *Puccinia graminis* f. sp. *tritici* pathotypes used to determine host response of South African wheat varieties.

NA code[a]	**UFS code**[a]	**SA code**[a]	**Avirulence/Virulence *Sr* genes**[b*]	**Selective hosts**[c]
BPGSC	UVPgt54	2SA55	*Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 27, 30, 31, 36, 38, Kw, Satu, Tmp*/*8a, 9a, 9b, 9d, 9g, 10, 11, McN*	McNair 701
TTKSF	UVPgt55	2SA88	*Sr9h, 24, 27, 31, 36, Kw, Satu, Tmp*/*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 30, 38, McN*	Barleta Benvenuto
BPGSC+*Sr27*	UVPgt57	2SA105	*Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 30, 31, 36, 38, Tmp*/*8a, 9a, 9b, 9d, 9g, 10, 11, 27, Kw, Satu, McN*	Kiewiet/Satu
TTKSP	UVPgt59	2SA106	*Sr9h, 27, 31, 36, Kw, Satu, Tmp*/*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 24, 30, 38, McN*	LCSr24Ag
PTKST	UVPgt60	2SA107	*Sr9h, 21, 27, 36, Kw, Satu, Tmp*/*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 24, 30, 31, 38, McN*	Fed4*/Kavkaz
TTKSF+*Sr9h*	UVPgt61	2SA88+*Sr9h*	*Sr24, 27, 31, 36, Kw, Satu, Tmp*/*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 9h, 10, 11, 17, 21, 30, 38, McN*	Matlabas
BFBSC+*Sr27*	UVPgt62	2SA108	*Sr5, 6, 7b, 8b, 9b, 9e, 11, 17, 21, 24, 30, 31, 36, 38, Kw, Satu, Tmp*/*8a, 9a, 9d, 9g, 10, 27, McN*	Coorong

[a]Pathotypes were named according to North American (NA) nomenclature (Roelfs and Martens, 1988). TTKSF, TTKSP, PTKST and TTKSF+*Sr9h* belong to the Ug99 lineage. TTKSF+*Sr9h* differ from TTKSF by its virulence on *Sr9h* (formerly *SrWeb*). University of the Free State (UFS) and South African (SA) codes

[b]Avirulence/virulence according to Terefe *et al*., 2016, Boshoff *et al*., 2018.

[c]Selective hosts on which pathotypes were increased. McNair 701 (*SrMcN*), Barleta Benvenuto (*Sr8b*), Kiewiet (triticale gene, *SrKw*), Satu (*SrSatu*), LCSr24Ag (*Sr24*), Federation4*/Kavkaz (*Sr31*), Matlabas (*Sr9h*), Coorong (*Sr27*)

Table 2.2.1: Typical seedling response observed to *Puccinia graminis* f. sp. *tritici* on South African wheat varieties and the three groups used to summarize the seedling infection types.

Resistant	Intermediate	Susceptible
;, ;1, ;12, 1++, 2=, 2-	2, 2+, 2++, 3=, 3- X	3, 3+, 3++, 4

Table 2.2.2: Adult plant host responses observed to *Puccinia graminis* f. sp. *tritici* on South African wheat varieties and their grouping into three reaction classes based on their calculated Coefficient of Infection.

Resistant – CI[a] <20	Intermediate – CI[a] 20 to 39	Susceptible – CI[a] >39
TR, 5R, 15R	50MR	50MS, 60MS, 70MS
10RMR, 15RMR, 20RMR, 30RMR, 50RMR	40MRMS, 50MRMS, 60MRMS	50MSS, 60MSS, 70MSS, 80MSS, 90MSS
TMR, 5MR, 10MR, 15MR, 20MR, 30MR, 40MR	30MS, 40MS	60S, 70S
5MRMS, 10MRMS, 20MRMS, 30MRMS	30MSS, 40MSS	
5MS, 10MS, 20MS	20S	
20MSS		

[a] CI = Coefficient of Infection where severity is multiplied with a constant for host reaction type: where R = 0.2, RMR = 0.3, MR = 0.4, MRMS = 0.6, MS = 0.8, MSS = 0.9 and S = 1

Table 2.3: Seedling infection types of 192 South African wheat varieties when inoculated with four different *Puccinia graminis* f. sp. *tritici* pathotypes in 2013.

		Seedling Infection Types			
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**
LINE1	Pannar	4	3++	4	4
LINE2	Pannar	1	;	1	;1
LINE3	Pannar	3+	2	3-	4
LINE4	Pannar	3	3+	3-	4
LINE5	Pannar	1	1	2	;1-
LINE6	Pannar	3+	1	3	4
LINE7	Pannar	;1	1	2	;1-
LINE8	Pannar	;1	;	1	;1-
LINE9	Pannar	;	;	1	0;
LINE10	Pannar	1	1	1	;1-
LINE11	Pannar	4	;	4	4
LINE12	Pannar	4	;	4	4
LINE13	Pannar	4	4	4	3
PAN 3623	Pannar	3+	3+	4	3
PAN 3478	Pannar	;1	3	4	3
PAN 3497	Pannar	3	3++	3	3
PAN 3515	Pannar	1	1	2	1
PAN 3489	Pannar	4	;, 12	4	4
PAN 3400	Pannar	4	3++	3	4
LINE20	Pannar	1	1	1+	;
LINE21	Pannar	;1	1	4	;1, 3
PAN 3471	Pannar	3	;	3	4
LINE23	Pannar	4	;	4	1
LINE24	Pannar	3	1	3+	4
LINE25	Pannar	3	1	4	4
LINE26	Pannar	1-	;	1	;1
LINE27	Pannar	4	4	3	4
PAN 3368	Pannar	2	;1	2	2
PAN 3379	Pannar	3++	;	4	3
LINE30	Pannar	4	;	3+;	3++
LINE31	Pannar	1	;	1	1
LINE32	Pannar	3, ;	;	nd	3
LINE33	Pannar	3	;	3	3
LINE34	Pannar	2+3	;	3	3++
LINE35	Pannar	2	;1	2	2
LINE36	Pannar	4	;	3+	3
LINE37	Pannar	2+3	;	2-	2+
LINE38	Pannar	2+	;	3-	3
PAN 3120	Pannar	3	;	3++	3++
PAN 3118	Pannar	3	;	3++	3++
PAN 3161	Pannar	4	;	3	4
PAN 3198	Pannar	3	;	3-	4

		Seedling Infection Types			
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**
PAN 3195	Pannar	3	;	3	4
PAN 3111	Pannar	2	;	3	3
LINE45	Pannar	3+	;	3	4
LINE46	Pannar	3-	;1	3	4
LINE47	Pannar	3	;	3	4
LINE48	Pannar	2	;1=	2	2
LINE49	Pannar	4	;	3	4
LINE50	Pannar	3	;	3	4
SST 822	Sensako	4	;	4	4
SST 843	Sensako	2+	;1=	2	2
SST 806	Sensako	3	1+	3	4
CRN 826	Sensako	3	3	3	4
SST 835	Sensako	3	2++	4	4
SST 867	Sensako	4	3	4	4
SST 866	Sensako	4	;	4	4
SST 877	Sensako	3+	;1	4	4
SST 875	Sensako	4	3	3	4
SST 895	Sensako	1	;1=	2+	;1
SST 884	Sensako	2	;1	1	;1-
SST 896	Sensako	1	;1	1, 3	2, 4
SST 805	Sensako	4	2	3++	4
SST 807	Sensako	2++	2	3++	4
SST 398	Sensako	;1	;	1	2
SST 316	Sensako	1	;	4	;1
SST 317	Sensako	0;	;	4	;1
SST 387	Sensako	2+	;	2	2
SST 347	Sensako	1	;	2	1
SST 356	Sensako	1	;	4	;1
SST 374	Sensako	4, 1	;	4	4
SST 015	Sensako	4	2+	3	4
SST 88	Sensako	4	3+	3++	4
SST 047	Sensako	0;	;	;	0;
SST 027	Sensako	1	;	2	2+3
SST 056	Sensako	4	;	4	4
SST 087	Sensako	1	;	1	;1
SST 096	Sensako	2	;	2+	;1
Elands	SGI	2	;1	2	2
T10/01	SGI	4	;1	3-	4
T10/02	SGI	4	;1	3	4
T10/03	SGI	3	2+	3+	3+
T10/04	SGI	3+	;	3-	4
T10/05	SGI	3	;	3+	4
T10/07	SGI	2	2	2	3+
T10/08	SGI	1, 2	;1	2	2+3

		Seedling Infection Types			
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**
T10/09	SGI	1	;	3	1
T10/10	SGI	1, 3	;1	1	3-, 1
T10/11	SGI	3	;1	1	4
T10/12	SGI	2+	;1	1	3
T10/13	SGI	4	;	4	4
T10/14	SGI	4	;	4	4
T10/16	SGI	2+	;	1	3-
T10/17	SGI	2++	2+	2	3
T10/18	SGI	1	1	1	;1
T10/20	SGI	;1-	;	nd	;1
T10/24	SGI	3	;, 1	3-	4
T12/00	SGI	3	;	3+	;1-
T12/01	SGI	1	;	;1	2
T12/02	SGI	1	;	;1	1
T12/03	SGI	2	;1	12	;12
T12/04	SGI	2	;1	2	2
T12/05	SGI	1	;	1	1
T12/06	SGI	1	;	1	1
T12/07	SGI	1	;1=	1	1
T12/08	SGI	1	;	4	1
T12/09	SGI	1	;	;1	1
T12/10	SGI	1	;	;1=	1
T12/11	SGI	2	;	1	4
T12/12	SGI	2+	;	2	2
T12/13	SGI	2	;1, 4	2	2
T12/14	SGI	4	;1	1, 4	1
T12/15	SGI	3-	;	3-	4
T12/16	SGI	3	;	3	3++
T12/17	SGI	3	;	3-	4
T12/18	SGI	4	;	4	4
T12/19	SGI	1	;	1	1
T12/20	SGI	;1	;	1	1
T12/21	SGI	3	2+	2+	3
T12/22	SGI	2	;	1	1
T12/23	SGI	2	;	;1	2
T12/24	SGI	1	;	4	1
T12/25	SGI	4	3++	4	4
T12/26	SGI	2+	2	1	2+3
T12/27	SGI	2+3	;	3-	4
T12/28	SGI	2+	;	2	2
T12/29	SGI	;1	;	1	1
T12/30	SGI	1	;	;1, 4	1, 3
T12/31	SGI	1	;	4	1
T12/32	SGI	1	;, 22+	1	1

		Seedling Infection Types			
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**
T12/33	SGI	1	;	4, 1	1
T12/34	SGI	3	;	;1	1
T12/35	SGI	1	;, 3	3-	1
T12/36	SGI	4	3+	3	4
T12/37	SGI	;1	;1=	1	2
T12/38	SGI	2+	;1	2	2+
Tugela DN	SGI	3++	;	3=	4
Senqu	SGI	1	;	1	1+
Koonap	SGI	2+	2	1	2
Matlabas	SGI	1	;	1	4
Elands	SGI	2	;1=	1	2
Gariep	SGI	2	;	1	12
Baviaans	SGI	4	3++	4	4
Buffels	SGI	4	3	3++	4
Duzi	SGI	;1	;1	3+	3+
Kariega	SGI	3	3	3+	4
Krokodil	SGI	4	3++	3	4
Kwartel	SGI	1	;1	2	;1
Olifants	SGI	4	;1	3+	4
Ratel	SGI	3++	;1	4	3++
Sabie	SGI	4	3-	3	4
Steenbras	SGI	0;	0;	0;	0;
Tambotie	SGI	4	4	3+	4
Tankwa	SGI	1	;	22-	;
Timbavati	SGI	3+	3++	4	4
Umlazi	SGI	4	4	4	4
BSP EL1	SGI	4	1	4	4
BSP EL2	SGI	4	4	4	4
BSP EL3	SGI	4	3+	4	4
BSP EL4	SGI	3+	4	4	4, 1
BSP EL5	SGI	1-	;1	2	;1, 4
BSP EL6	SGI	2+3	3	3++	4
BSP EL7	SGI	4	;	3++	4
BSP EL8	SGI	1	;1	1	;1
BSP EL9	SGI	3	X=	4	4
BSP EL10	SGI	4	2++	4	4
BSP EL11	SGI	4	3+	4	4
BSP EL12	SGI	4	;1, 3	4	4
BSP EL13	SGI	;1, 4	2+3	2, 3	;1, 4
BSP EL14	SGI	4	4	4	4
BSP EL15	SGI	4	;1	4	4
BSP EL16	SGI	3+	3	4	4, 0
BSP EL17	SGI	3	3++	3	4
BSP EL18	SGI	3, 1	3++	3	4

		Seedling Infection Types			
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**
BSP EL19	SGI	3	3	3++	4
BSP EL20	SGI	;1=, 3	3	3+	4, 0
BSP EL21	SGI	3++	3	3+	4
BSP EL22	SGI	;1=	;1	nd	;1-
BSP EL23	SGI	;1	;1	1, 3	1
BSP EL24	SGI	4	1	3	3
BSP EL25	SGI	3	;	4	4
BSP EL26	SGI	4	;	4	4
BSP EL27	SGI	0;	;	0	0;
BSP EL28	SGI	3	2+	3	4
BSP EL29	SGI	3++	3++	4	4
BSP EL30	SGI	3+	;	3+	3++
BSP EL31	SGI	3	;, 1	3	4
BSP EL32	SGI	4	;	4	4
BSP EL33	SGI	0;	;	0;	0;
BSP EL34	SGI	;	;	0;	;1=
BSP EL35	SGI	3	;	3	3++
BSP EL36	SGI	3	;1-	3-	4
Line 37-07*		4	4	3+	4
Tobie*		;	4	0;	;
Sr31 line*		;	2-	4	;1
Morocco*		4	4	3++	4
McNair 701*		4	3+	3+	3+

Mixed infection type scores: e.g. ;1, 4 where most plants had a score of ;1 and a lesser amount had a score of 4.

nd: no data

*Checks: Seedlings - Tobie (*Sr27, SrSatu*), Federation 4/Kavkaz (*Sr31*), Morocco and McNair 701 (*SrMcN*) (universal susceptibles)

Adult plants - Line 37-07 (susceptible)

Table 2.4: Seedling infection types and adult plant severity and reaction types of 122 South African wheat varieties when inoculated with four different *Puccinia graminis* f. sp. *tritici* pathotypes in 2014.

		Seedling Infection Types				Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
PANLE1	Pannar	4	3	3	3++	10MR
PANLE2	Pannar	1=	;	2+	1	60MSS
PAN 3400	Pannar	4	3	3	4	5MS
PAN 3623	Pannar	3	3	4	4	30MS
PANLE5	Pannar	;1=	1-	2	1	20S
PAN 3489	Pannar	4	1	3+	3	40MR
PAN 3471	Pannar	3	;	3+	4	40MR
PANLE8	Pannar	4	;	3++	4	50MR
PANLE9	Pannar	3	1	3	3	30MR
PANLE10	Pannar	3++	2	4	3++	40MRMS
PANLE11	Pannar	1=, 3	;	2+	1, 4	40MRMS
PAN 3515	Pannar	1	1	2+	1	80MSS
PAN 3497	Pannar	4	3	3++	4	20MR
PANLE14	Pannar	4	1	3, 0	4	40MR
PANLE15	Pannar	4	2	3++	4	10MR
PANLE16	Pannar	3++	2	4	4	15MR
PANLE17	Pannar	3	;	3++	4	40MR
PANLE18	Pannar	4	2	4	4	15MR
PANLE19	Pannar	1	;	2	1	25MS
PANLE20	Pannar	4	;	4	4	20MRMS
PANLE21	Pannar	3-	1	3+	4	15MR
PANLE22	Pannar	4	3-	4	2, 3	10MRMS
PANLE23	Pannar	;1=, 1, 4	;	2++	;1, 3	50MSS
PANLE24	Pannar	;1	1	2++	1	60MSS
PANLE25	Pannar	4	2	4	4	30MR
PANLE26	Pannar	;, ;1	;	2	;1	60MRMS
PANLE27	Pannar	1	1	2	1	20MS
PANLE28	Pannar	4	2	3+	4	20MR
PANLE29	Pannar	;	1	2++, 0	1	50MSS
PAN 3368	Pannar	2+	;1	1	2	20R
PAN 3379	Pannar	3	;	3++	3++	50MSS
PANWE32	Pannar	3	;	3++	4	40S
PANWE33	Pannar	3++	;	2+	;1	5R
PANWE34	Pannar	3++, 1	1	nd	4	40MSS
PAN 3120	Pannar	3++	;	3	3	30MRMS
PAN 3118	Pannar	4	0;	4	3	20RMR
PAN 3161	Pannar	4	0;	3	4	0R
PAN 3198	Pannar	3++	0;	3-	3	TMR
PAN 3195	Pannar	3++	0;	3	4	40MRMS
PAN 3111	Pannar	4	0;	3	3++	TMR
PANEW41	Pannar	4	0;	3	4	0R
PANEW42	Pannar	4	0;	2++	4	5R

		Seedling Infection Types				Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
PANEW43	Pannar	4	0;	3-	4	20MRMS
PANEW44	Pannar	3++	0;	3+	2++	TR
PANEW45	Pannar	4	0;	1	2++	TMR
PANEW46	Pannar	3++	2+	2+	2+	0R
PANEW47	Pannar	4	0;	3++	3	0R
PANEW48	Pannar	4	0;	4	3	25MS
PANEW49	Pannar	2	0;	1-	0, 4	10RMR
PANEW50	Pannar	4	0;	3	4	40MSS
SST 88	Sensako	4	3	4	4	70S
SST 843	Sensako	2++	1	2	2+	70S
SST 806	Sensako	4	;1	3++	3++	70MSS
SST 835	Sensako	4	1	3+	4	80MSS
SST 866	Sensako	4	0;, 1	3+	3	40MSS
SST 867	Sensako	4	3	3++	3+	70MSS
SST 877	Sensako	4	;	3	4	60MRMS
SST 875	Sensako	4	3	4	4	80S
SST 884	Sensako	1	1	1	1	80MSS
SST 895	Sensako	1	;1	3=	1	100S
SST 896	Sensako	1	1	2	1	40MRMS
SST 347	Sensako	1	;	2	1	50MS
SST 356	Sensako	;1=	;	4	1	70S
SST 387	Sensako	2+	;	1	2+	TMR
SST 374	Sensako	4	;	4	3++	70MSS
SST 398	Sensako	12	;	1	1	40RMR
SST 316	Sensako	;1-	0;	3++	1	80MRMS
SST 317	Sensako	1	0;	4	1, 3	60MRMS
N538/1	Sensako	3	1p 3	3	2	nd
N539L/6	Sensako	1	0;	4	;1	nd
N539L/9	Sensako	nd	0;	4	2+	nd
N539L/10	Sensako	nd	0;	X	3	nd
T08/21	SGI	2++	0;	1	3, 1	20R
T09/24	SGI	3	3-	1	1, 3	5R
T10/03	SGI	3	2	3+	4	40S
T10/07	SGI	1	0;	1	3+	10R, 80S
T10/08	SGI	2	2	1	2++	15R
T10/09	SGI	;1=	0;	3+	2	20R
T10/11	SGI	3	0;	1	4	10R
T12/05	SGI	2	0;	1	1	5R
T12/06	SGI	2	0;	1	1	20R
T12/07	SGI	2	;1=	1	1	40RMR
T12/24	SGI	2	0;	3	1	60S
T12/32	SGI	2	1	1	2-	20RMR
T12/34	SGI	3	;	1	1	0R, 5MS
T13/01	SGI	3++	0;	4	4	50MSS
T13/02	SGI	3++	0;, 3+	4	4	20MS

		Seedling Infection Types				Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
T13/03	SGI	4	2++	4	4	40MSS
T13/04	SGI	;1=n	0;	2+	1	nd
T13/09	SGI	2++	2	2++	4	70MSS
T13/10	SGI	;1	0;	1	1	5R
T13/14	SGI	4	0;	3	4	15S
T13/15	SGI	3-	0;	1	4	10R
T14/01	SGI	2	;1=	1	2, 3	20R
T14/02	SGI	2	3	2	3-	10R
T14/03	SGI	4	X	2	4	30RMR
T14/04	SGI	4	0;	4	4	60MSS
T14/05	SGI	4	2+	1	2+	20R
T14/06	SGI	4, 1	;1=, X	4	1, 4	40MRMS
T14/07	SGI	4	0;	4	3	TR
T14/08	SGI	4	0;	4	4	TR
T14/09	SGI	4	2	3+	3-	90S
T14/10	SGI	4	2+	3+	4	50MS
T14/11	SGI	4	;	3++	4	30MR
Elands	SGI	2+	;1=	1	2	20R
Gariep	SGI	2	;1=	1	1	10R
Koonap	SGI	2-	1	1	2	10R
Matlabas	SGI	2+	0;	2	4	5R
Senqu	SGI	2++	;1=	1	1	15R
Buffels	SGI	4	3	3++	4	70MS
Duzi	SGI	;1	1	3-	3+ ;1	10R, 50MSS
Krokodil	SGI	4	3++	4	4	30MRMS
Sabie	SGI	3	2++	3	4	50MS
BSP EL1	SGI	4	2+	4	4	nd
BSP EL2	SGI	3-	2++	4	4	nd
BSP EL3	SGI	4	2++	4	4	nd
BSP EL4	SGI	4	2++	4	3+	nd
BSP EL5	SGI	;1	;1=	2	;1	nd
BSP EL6	SGI	3+	2+	3	4	nd
BSP EL7	SGI	4	0;	4	4	nd
BSP EL8	SGI	1	1	2	1	nd
BSP EL9	SGI	4	1	4	3	nd
Line 37-07*		4	4	4	4	100S
Satu*		;	4	0	;	na
Sr31 line*		;1	1	4	;1	na
Morocco*		4	4	4	4	na

Mixed infection type scores: e.g. 1, 4 where an equal split is found.
nd: no data
na: not applicable
*Checks: Seedlings - Satu (*SrSatu*), Federation 4/Kavkaz (*Sr31*) and Morocco (universal susceptible)
Adult plants - Line 37-07 (susceptible)

Table 2.5: Seedling infection types and adult plant severity and reaction types of 145 South African wheat varieties when inoculated with five different *Puccinia graminis* f. sp. *tritici* pathotypes in 2015.

		Seedling Infection Types					Field trial Greytown
Entry	**Origin**	**UVPgt54**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
PAN 3402	Pannar	0;	3	0;	2++	3	70MR
PAN 3596	Pannar	2+	4	3++	4	4	60MR
PAN 3494	Pannar	;	;	0;	22+	1-	50MR
PANLE4	Pannar	1	2+	2	3	2++	40MR
PANLE5	Pannar	1	3-	2	3++	3-	60MR
PANLE6	Pannar	;	;1	;	2	1	80MS
PANLE7	Pannar	;	;1	;	2+	;1	60MRMS
PANLE8	Pannar	;1	;	;1	2	;1	40RMR
PANLE9	Pannar	2	2	1	3++	4	60MS
PANLE10	Pannar	;	;1	;	2	;1	50MS
PANLE11	Pannar	;	1	;	2+	1	40MRMS
PANLE12	Pannar	;1	;1	;	2+	;1	50MS
PANLE13	Pannar	;1-	;1-	;	2	;1	80MS
PANLE14	Pannar	1	;1-	;	3-	;1-	70MRMS
PAN 3408	Pannar	2	2+	1	3-	4	70MRMS
PANLE16	Pannar	2	3, ;1	2++	3-	3+	80MRMS
PANLE17	Pannar	;	2+	;	2+	2++	70MRMS
PANLE18	Pannar	2	2	2	3-	3-3	60MRMS
PANLE19	Pannar	1	2+	1	3-	3	70MRMS
PANLE20	Pannar	1	;1	1	3-	1-	40MRMS
PANLE21	Pannar	;	3	;	3++	4	70MS
PANLE22	Pannar	2+	2+3	;1	4	3	80MS
PANLE23	Pannar	2	3	2++	4	3	70MR
PANLE24	Pannar	1	;	1	1	;1	70MRMS
PANLE25	Pannar	;	;1	;	1	1	70MS
PANLE26	Pannar	;1	;1	;	2++,1p4	1, 3++	70MS
PANLE27	Pannar	2	3-	2	3-	3	60MR
PANLE28	Pannar	;1	3-	;	3++	3	70MS
PANLE29	Pannar	2	3-	2	3	4	60MRMS
PANLE30	Pannar	1, 3	;1	1	2	4, 1	80MS
PAN 3368	Pannar	;1=	1	1	1	1	80MR
PAN 3379	Pannar	;	3	;	3	3+	80MRMS
PAN2013_8	Pannar	2-	4	;1	3	4	50MRMS
PAN 2014_2	Pannar	;	2, 4	;	4	4	50MRMS
PAN2014_3	Pannar	;	0	;	3+	1p4	50MS
PAN2014_22	Pannar	;	1p3	;	3-	3	100MRMS
PAN2014_23	Pannar	;	2+	;	3+	4	40MS
PAN2014_24	Pannar	;	1	;	3	3	5MR
PAN3120	Pannar	;	3	;	3-	4	15MRMS
PAN3118	Pannar	;	4	;	3+	4	20R

		Seedling Infection Types					Field trial Greytown
Entry	**Origin**	**UVPgt54**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
PAN3161	Pannar	;1=	4	;	4	4	TR
PAN3198	Pannar	;	3++	;	3-	4	5R
PAN3195	Pannar	;	1p3	;	3	3+	30MR
PAN3111	Pannar	;	3	;	3	3	TR
PAN3133	Pannar	;	3-	;	3+	3++	10MRMS
PAN2013_5	Pannar	2	1	0;	2	1p2++	20RMR
PAN2014_26	Pannar	;	2+	0;	3	3	50MRMS
PAN2014_35	Pannar	;	2	0;	3	3	50MRMS
RobL1	SGI	;	;1-	;	2++	1-	80MS
RobL2	SGI	;	;1	0;	2++	1	90MS
RobL3	SGI	;	2, ;	;	2+	;1, 1p4	90S
RobL4	SGI	;	1	1-	2+	;1=	70MRMS
RobL5	SGI	0;	3	0;	3	4	30MR, 80S
RobL6	SGI	1	;1	1=	1	;1-	50MR
RobL7	SGI	;	;1	;	1	;1-	40RMR
RobL8	SGI	;1	3+, ;1	;1=	4	3+	80S
RobL9	SGI	;	3	;	4	4, 1p1	90S
RobL10	SGI	;1=	;1	;1	2	1, 1p2+	70MSS
RobL11	SGI	;	;1	;	2	1	60MS
RobL12	SGI	;	;1	;	2	;1=	60MS
W11/04	SGI	;	;1	;	2p1, 2p4	1, 4	80MSS
W11/09	SGI	0;	;1-	0;	2+	1	100MSS
W12/12	SGI	;	;1=	0;	3	1	40MR
Ratel	SGI	1	3++	1+	4	3++	40MR
Kwartel	SGI	;1	;1	1	22+	1	70MSS
Tankwa	SGI	0;	1	0;	1	1-	60MRMS
Krokodil	SGI	2	3+	3+	4	4	60MRMS
BSP EL1	SGI	22+	3-	2	3++	3-	70MS
BSP EL2	SGI	2	2++	2	2++	3	80MS
BSP EL3	SGI	1, 2++	1	1	2+	1-	80MSS
BSP EL4	SGI	2	3	2	3+	3	80MS
BSP EL5	SGI	0;	;	0	4	4	40MR
BSP EL6	SGI	1	;1-	1	2	1-	80MRMS
BSP EL7	SGI	2++	2+	2+	3++	3-	70MR
BSP EL8	SGI	2	2++	2++	4	4	nd
BSP EL9	SGI	2+	2	3++	4	3++	90MS
BSP EL10	SGI	;1	;1	1	4	3+	90MSS
BSP EL11	SGI	2	2	2	2++	4	90MR
BSP EL12	SGI	2+	2+	2+	4	4	70MRMS
BSP EL13	SGI	1	1	1	2	1	40MR
BSP EL14	SGI	;	2	;	4	4	50MR
BSP EL15	SGI	;	0	;, 2	0;	0	5R
BSP EL16	SGI	;	3	;	4	3+	30MR

		Seedling Infection Types					Field trial Greytown
Entry	**Origin**	**UVPgt54**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
BSP EL17	SGI	2	2++	1	4	4	nd
Matlabas	SGI	0;	1	0;	1	4	80RMR
Gariep	SGI	;1=	;1	;	1	1-	60R
Elands	SGI	;	1	;	1	1	80RMR
Hartbees	SGI	2	2+	;	2++	;1	70R
Selati	SGI	;1=	1	;	1	1	90RMR
Senqu	SGI	;1-	;1	;	1	1+	80RMR
Koonap	SGI	1	1	1	1	2	70R
Kouga	SGI	1	1	;	2, 1p3	2, 2p4	50MRMS
Shingwedzi	SGI	;1	1	;	4	3+	70MRMS
T08/21	SGI	;;1	2+	;	1	4, 1	80R
T13/04	SGI	;	;1=	;	2	;1=	15RMR
T13/09	SGI	2	2	1	2++	3++	10MSS
T10/11	SGI	;1=	4	1	2	4	80RMR
T10/03	SGI	;1	3	;1	3+	3	70MS
T10/08	SGI	;1, X	1	;1	1	2+	80MRMS
T13/10	SGI	0;	;1-	0;	2	1	40R
T13/15	SGI	;	2	;	1	4	30R
Line 37-07*							100S
							Makhathini
SST 316	Sensako	0;	;1	;	4	1	15MS
SST 317	Sensako	0;	;1	0;	4	1	5MR
SST 347	Sensako	;1=	1	0;	1+	1	TR
SST 356	Sensako	0;	1	0;	3++	1	TR
SST 374	Sensako	0;	4	0;	4	4	20MRMS
SST 387	Sensako	;	;1	0;	1-	1	20S
SST 398	Sensako	;n	1	;n	1	1+	0R
SST 3127	Sensako	;1-	;1	;1	1	1	15MRMS
SST 3149	Sensako	1	;1	1	1	1	TR
SST 3156	Sensako	0;	1	;	1	1	TMR
SST 806	Sensako	2	3	2	4	4	5MS
SST 835	Sensako	2	3	2++	3	3+	nd
SST 843	Sensako	1	;1	1, 1p4	1	1	nd
SST 866	Sensako	;1	4	2	3++	3	TMR
SST 867	Sensako	2, 2++	4	3	4	3	TMS
SST 875	Sensako	3	4	3	4	4	nd
SST 877	Sensako	2	4	2++	4	2	5MR
SST 884	Sensako	1	1	1	1	1	TMR
SST 895	Sensako	1	1	1	2	1	10MRMS
SST 896	Sensako	1	1	1	2	1	5MRMS
SST 8125	Sensako	2	3	3+	4	4	15MSS
SST 8134	Sensako	;1, 2++	3+	;	3++	3	TMRMS
SST 8135	Sensako	1	3+	1	4	1	TMR

		Seedling Infection Types					Field trial Greytown
Entry	**Origin**	**UVPgt54**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
SST 8145	Sensako	2	3+	2	4	3+	TMS
SST 8154	Sensako	2	2	2	3++	3++	0R
SST 8155	Sensako	2++	2++	2	4	3+	0R
SST 8156	Sensako	23	3	2	3	3++	TMR
SST 88	Sensako	4	4	3++	4	4	40S
SST 015	Sensako	2	3++	1	4	4	5MR
SST 027	Sensako	;	2	;	2	2++	TMR
SST 056	Sensako	2	1, 3-	2	4	4	60S
SST 087	Sensako	;	;1	;	2	1	20MS
SST 096	Sensako	;	;1, 4	;	2+	1	40S
SST 0127	Sensako	0;	3	;	4	4	20MRMS
SST 0147	Sensako	0;	1	0;	2+	1	5MR
CB15-03	Sensako	2+	2++	2++	3+	3++	nd
CB15-13	Sensako	23	4	3	4	3+	nd
CB15-17	Sensako	0;	;1-	;	1	1	nd
CB15-18	Sensako	;	;1	;	1	1	nd
CB15-19	Sensako	;	;1	;1=	2	1	nd
CB15-20	Sensako	;	;1-	0;	2?	;1	nd
CB15-21	Sensako	;	;1	0;	1p3=	1	nd
CB15-22	Sensako	;	;1	0;	3++	1	nd
Line 37-07*		na	na	na	na	na	70S
Satu*		na	na	4	na	na	na
Sr31 line*		1	1	1	4	1	na
Morocco*		4	4	4	4	4	na
McNair 701*		4	4	4	4	4	30S
Kiewiet*		na	na	2, 1p4	na	na	na

Mixed infection type scores: e.g. 2++,1p4 where most plants had a score of 2++ while a single plant had a score of 4 or 3, ;1 where an equal split is found.
nd: no data
na: not applicable
pi: poor infection
*Checks: Seedlings - Satu (*SrSatu*), *Sr31*(Benno)/6*LMPG-6 (*Sr31*), Morocco and McNair 701 (*SrMcN*) (universal susceptibles)
Adult plants - Line 37-07 (susceptible)

Table 2.6: Seedling infection types and adult plant severity and reaction types on 116 South African wheat varieties when inoculated with five different *Puccinia graminis* f. sp. *tritici* pathotypes in 2016.

		Seedling Infection Types					Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt62**	**UVPgt60**
SHINE	Pannar	;1	0	2	;1	;	70MR
PAN 3402	Pannar	3	0	2	3+	;1	40MR
PAN 3596	Pannar	4	3++	3+	4	2++	60MR
PAN 3494	Pannar	;1	0	1+c2=	;1	;	60MR
ZIMZAM2012/9	Pannar	2+	;1	3-	3++	1	70MR
ZIMZAM2014/8	Pannar	3	1	3	3	1	50MR
ZIMZAM2014/9	Pannar	;1	0;	2	;1	;1-	50MR
ZIMZAM2014/18	Pannar	;1	;1	2	;1	1+	50MR
ZIMZAM2014/19	Pannar	;1	;1	1c	;1	1	70MR
SST 806	Pannar	3	2	3	3	;1	50MS
PAN 3400	Pannar	3	3-	3	3	2	40MRMS
PAN 3453	Pannar	3=	0;	1, 1p2	2++	;	80MS
PAN 3471	Pannar	3	;	3	3++	;	80MS
PAN 3497	Pannar	4	3+	3++	4	nd	40MR
PAN 3515	Pannar	;1	1	1+	;1	;	90MS
PAN 3541	Pannar	3++	;	3	4	0;	90MRMS
PAN 3555	Pannar	;1	;	1+	;1	0;	90MR
PAN 3623	Pannar	4	3	4	3	3	90MRMS
PAN 3644	Pannar	3	3	3+	3	3+	20R
PANLE8	Pannar	2++	0;	3+	3	;	80MRMS
PANLE35	Pannar	3++	;1-	4	4	0;	90MRMS
PANLE36	Pannar	3=	;1	3+	4	;1=	60MR
PANLE42	Pannar	;1	0;	1p1++	1	0;	50MRMS
PANLE45	Pannar	2+	0;	3-	3	;	60MRMS
Elands	Pannar	1	;1	1c	;1	;	50RMR
PAN 3368	Pannar	2	;1	1c	2++	;1	70RMR
Matlabas	Pannar	1	0;	1c	4	0;	70RMR
PAN 3118	Pannar	3	0;	4	4	0;	40MR
PAN 3161	Pannar	3++	;1	4	4	0;	TR
PAN 3111	Pannar	3	0;	3	3	0;	TR
PAN 3133	Pannar	3++	0;	4	4	0;	5R
PAN 3252	Pannar	2	3++	3-	4	3	5R
PAN2013-8	Pannar	3+	;	4	4	;	40MS
PAN2015_1	Pannar	3=	0;	4	4	0;	20MRMS
PAN2015_5	Pannar	3+	0;	4	3++	0;	50MRMS
PAN2015_14	Pannar	3	0;	3	4	;1	TR
PAN2015_19	Pannar	3	0;	4	3	;	10MR, 60S
PAN2015_20	Pannar	3	0;	4	2+	;	60MS
PAN2015_22	Pannar	2+	2+	2	2	2	20RMR
PAN2015_23	Pannar	4	;1	4	4	;1	TR
PAN2015_24	Pannar	4	0;	3	4	0;	TR
PAN2015_31	Pannar	4	0;	X	4	0;	5R

		Seedling Infection Types					Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt62**	**UVPgt60**
SST 806	Sensako	3-	1+	3++	3++	;1-	50MS
SST 835	Sensako	3-	2	4	4	2	30MR
SST 843	Sensako	1+	;1	2	2-	;1	15MR
SST 866	Sensako	3-	;1	3	4	;1	10MR
SST 867	Sensako	3	3+	3+	4	3	10RMR
SST 875	Sensako	3++	3++	3+	4	3	10MR
SST 877	Sensako	4	;1	4	4	1	30MR
SST 884	Sensako	;1=	;1	1	;1=	;1	20MR
SST 895	Sensako	;1	;1	1	;1	1	30MR
SST 896	Sensako	;1	;1	1	;1=	;1	20MR
SST 8125	Sensako	3++	3+	3++	4	1	30MR
SST 8135	Sensako	;1=	1	3+	;1	1	20MRMS
SST 8154	Sensako	2+	3-	4	3-	2	20MR
SST 8155	Sensako	2++	3-	3+	3	2	TR
SST 347	Sensako	0;	0	0	0;	;	0
SST 356	Sensako	0;	0	3++	0;	;	20MR
SST 374	Sensako	4	0	4	4, 1	0;	70MS
SST 387	Sensako	1	0	1	2+	;	0
SST 398	Sensako	;1	0	;1	;1	;1=	20R
SST 316	Sensako	;1=	0	4	;1	0;	30RMR
SST 317	Sensako	;1=	0	4	2+	0;	30MR
SST 3149	Sensako	1=	1	2	;1	;1	TR
SST 88	Sensako	4	3-	4	4	2	60S
SST 015	Sensako	4	;1	3c	4	;1	20RMR
SST 027	Sensako	3	0;	2-	2	;	10RMR
SST 056	Sensako	3	2	4	4	;,2	5RMR
SST 087	Sensako	1	;1=	1+	;1	;1=	80MRMS
SST 096	Sensako	1, 4	;	1++	;1	;1=, 1p2	30MRMS
SST 0127	Sensako	3	;	4	3	;	15MRMS
SST 0117	Sensako	4	3++	4	4	3	60MS
SST 0147	Sensako	1	0	1	1	0;	10RMR
SST 0137	Sensako	0;	0;	0;	;	0;	50MS
LNR ent. 1	SGI	3	;1	3p2,1p2++	3+	1	80MR
LNR ent. 2	SGI	2+	2+	1++	3	1	100MSS
LNR ent. 3	SGI	;1c, 2+	;1	2p2, 2pX	;1	1+	90MRMS
LNR ent. 4	SGI	2++	2++	2++	3	3-	100MS
LNR ent. 5	SGI	2++	0;	2	3	0;	TMR
LNR ent. 6	SGI	;1	1	1	;1	0;	100MSS
LNR ent. 7	SGI	3-	;	3	4	;1	80MRMS
LNR ent. 8	SGI	3+	3-	3	4	;1	70MR
LNR ent. 9	SGI	1	;1	2-c	1	1	TR
LNR ent. 10	SGI	2++	0;	3++	4	;	10R, 10MS
LNR ent. 11	SGI	3+	;1	3	4	0;	10R
LNR ent. 12	SGI	3	2+	3+	4	;1	5R
LNR ent. 13	SGI	;1	1	1	;1=	1	100MS

		Seedling Infection Types					Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt62**	**UVPgt60**
LNR ent. 14	SGI	2++	0;	3	3+	0;	20MR
LNR ent. 15	SGI	;1=	1	1, 1p2++	;1=	;1	100MS
LNR ent. 16	SGI	2++, ;	1	3	4	2	100MSS
LNR ent. 17	SGI	3	0;	3++	4	;1-	70MR
LNR WRS 1	SGI	1, 3	;	1,4	;1	1	80MS
LNR WRS 2	SGI	;1=	;	1	;1=	;	50MR
LNR WRS 3	SGI	;1=	;	1	;1	;	40RMR
LNR WRS 4	SGI	;1	1	1	;1	nd	90MR
LNR WRS 5	SGI	;1	;1	1	;1	1	30MR
LNR WRS 6	SGI	;1	0;	1, 2p3	;1	;1	90MRMS
LNR WRS 7	SGI	3++	0;	3=	3++	;	30MRMS
LNR WRS 8	SGI	4	3-	4	4	3	80MRMS
LNR WRS 9	SGI	;1=	;1	2	;1=	1	90MS
LNR WRS 10	SGI	;1	;	3+	;1=	;	60MR
LNR WRS 11	SGI	1	;	3++	;1=	;1	30MR
LNR WRS 12	SGI	2++	0;	2-	3++	;1	90MS
LNR WRS 13	SGI	;1=	0;	1++	;1=	0;	50MR
Matlabas	SGI	1+	0;	1+	4	0;	40RMR
Kouga	SGI	2	0;	1, 1p3	3-	;	20R
Shingwedzi	SGI	2+	;	4	3	;	10R
T08/21	SGI	1+	0;	1+	3++	0;	30R
T13/04	SGI	;1n	0;	1+	;1n	0;	5R
T13/09	SGI	2	1+	2++	3	1+	50MS
T14/09	SGI	2	2	3+	2++	2	90S
T14/10	SGI	2	2	3+	4	2	90S
T15/01	SGI	2	0;	1	2++	0;	50RMR
T15/02	SGI	4	0;	4	4	0;, 1p3	80S
T15/06	SGI	2	0;	1	2, 4	0;	40RMR
T15/07	SGI	2+	0;	1++	2++	0;	40RMR
T15/08	SGI	1	1	1++2	2	2	40RMR
T15/09	SGI	2++	0;	1	2+	;1	70MS
T15/10	SGI	2	;	1+	2	;	60RMR
T15/11	SGI	2+	0;	;1	3++	0;	30RMR
T15/12	SGI	;1-	0;	1	2	0;	30RMR
Line 37-07*		na	na	na	na	na	90S
Barleta*		4	X-	4	4	X	na
Satu*		0;	3	;	;	;	na
Sr31 line*		1	1	4	1	1	na
Matlabas*		1+	0	1+c	4	0;	na
Coorong*		;	3	;	;	3	na

Mixed infection type scores: e.g. 1,1p2 where most plants had a score of 1 while a single plant had a score of 2 or 4,;1= where an equal split is found.
nd: no data
na: not applicable
*Checks: Seedlings - Barleta Benvenuto (*Sr8b*), Satu (*SrSatu*), Federation 4/Kavkaz (*Sr31*), Matlabas (*Sr9h*), Coorong (*Sr27*)
Adult plants - Line 37-07 (susceptible)

Table 2.7: Seedling infection types and adult plant severity and reaction types on 116 South African wheat varieties when inoculated with four different *Puccinia graminis* f. sp. *tritici* pathotypes in 2017.

		Seedling Infection Types				Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
PAN 3400	Pannar	2+3	3	3	3	50MSS
PAN 3453	Pannar	;1	;1	2+	2+	20MS
PAN 3471	Pannar	3	;	3	3	50MS
PAN 3497	Pannar	2	3	3+	3+	30MS
PAN 3515	Pannar	;1	1+	2-	;	30MRMS
PAN 3541	Pannar	3	;	3	3	40MS
PAN 3555	Pannar	;1	;	2	;1	40MS
PAN 3623	Pannar	3	3	3+	3	60MS
PAN 3644	Pannar	3+	3	3	3+	40MS
2015PANLE35	Pannar	3	;1	2+	3	30MS
2015PANLE42	Pannar	;1n	0;	12	;	20MR
2015PANLE45	Pannar	;1	1n	2-	;	30MSS
2016PANLE5	Pannar	2	;1	2-	;12+	15MS
2016PANLE12	Pannar	3+	;	2	;, 3	20MSS
2016PANLE15	Pannar	1	;1	2-	;	20MSS
2016PANLE16	Pannar	3+	1+	2++	3	20MS
2016PANLE24	Pannar	3+	;1	2++	3+	20MR
2016PANLE29	Pannar	22+	1	3	22+	10MRMS
PAN 3408	Pannar	22+	2=	2+	3	20MS
PAN 3478	Pannar	2	3	4	3	40MSS
PAN 3489	Pannar	4	1+	4	4	10MR
PAN 3368	Pannar	2-	0;	1	2-	50MR
PAN 3161	Pannar	3+	0;	3	3+	15RMR
PAN 3111	Pannar	3	0;	3	3	20RMR
PAN 3133	Pannar	3+	0;	3+	3++	30MRMS
PAN2015_20	Pannar	3	;12	2++	3	30RMR
PAN2015_23	Pannar	3X	;1	2++3	3+	15RMR
PAN2016_4	Pannar	nd	;	2+	3	30MS
PAN2016_8	Pannar	1p2-	0	3	3	30MS
PAN2016_11	Pannar	3	;	2+	3	30MRMS
PAN2016_13	Pannar	3	0;	3	3	30MRMS
PAN2016_16	Pannar	1	;n	3	3	15R
SST 88	Sensako	4	3	4	3+	20S
SST 015	Sensako	3+	;,12,3	4	3+	10MRR
SST 027	Sensako	2	;	12+	2	10MR
SST 056	Sensako	3	12	4	3	10MS
SST 087	Sensako	;1	;1=	22+	;1	5MR
SST 096	Sensako	;1, 3+	;1=	3+	;1	10MR
SST 0117	Sensako	3++	;,1p3	4	3+	20MS
SST 0127	Sensako	22++	;	4	3	10MRR
SST 0147	Sensako	;1	;1=	1	;	10MR

		Seedling Infection Types				Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
SST 0166	Sensako	;1	;1=	2-	;	30MS
CRN 826	Sensako	3	2=	3	3	30MRMS
SST 822	Sensako	3+	;	3	3+	20MR
SST 876	Sensako	3	3	3	3	10MR
SST 806	Sensako	3	12	3	3	20MR
SST 8125	Sensako	3+	2	3	3	15MR
SST 8135	Sensako	3+	;1	3	1p;	20MS
SST 8154	Sensako	3+	2++	3	3-	30MRMS
SST 8155	Sensako	3+	2++	3	3	TMR
SST 8156	Sensako	3	12+	3	3	20MRMS
SST 835	Sensako	3+	2+	3	3	20MRMS
SST 843	Sensako	2	1	1	;1	15MR
SST 866	Sensako	3+	;1	3	3	15MR
SST 875	Sensako	3+	3	3	3	20MRMS
SST 877	Sensako	3+	;1-	3	3+	30MRMS
SST 884	Sensako	1	1-	2	;	10MS
SST 895	Sensako	1	1	2-	;	15MR
SST 896	Sensako	;1	1	1	;	10MRMS
SST 7156	Sensako	4	;12+	4	3+	30MRMS
SST 7157	Sensako	4	;1 ,1p3	4, 1p2+	3++	20MRMS
SST 8134	Sensako	3+	;12	3	3	15MRMS
SST 8136	Sensako	3+	2	3+	3+	20MR
SST 8145	Sensako	3+	12	3	3+	30MRMS
SST 8176	Sensako	3++	;12, 3	3, 2p0	3+	20MR
SST 8175	Sensako	3	12	1, 2++	3	20MR
SST 3149	Sensako	1	;12	1	;1	40MRMS
SST 3156	Sensako	;1	0	;1	;1	40MR
SST 316	Sensako	;	0	4	;1	30MS
SST 317	Sensako	;1	;1	4	;	30MS
SST 347	Sensako	;1	0;	2-	;1	40MRMS
SST 356	Sensako	;1-	0	3	;1	50MS
SST 374	Sensako	;, 4	0;	3+	3+	60MSS
SST 387	Sensako	;1	0;	;1	;1	40MR
SST 398	Sensako	;1-	;n	1n	;1	50MR
Ratel	SGI	3	12	3+	3	20MRMS
Steenbok	SGI	;1	;1	2+	;	5MS
Tredou	SGI	;12, 1p2+3	;1-	1	;, 1p2++	30MS
Tankwa	SGI	;	0;	2	;	5MRMS
W17/01	SGI	;1	;1-	12+	;1	TMR
W17/02	SGI	2+	;1, 2+	1++	3+	10MR
W17/03	SGI	;1	;1-	3+	;	30MSS
W17/04	SGI	3	;1+	2++	3+	10MR
W17/05	SGI	;	;1-	1	;	40MS
W17/06	SGI	;, 1p3	nd	1	0;	20MS

		Seedling Infection Types				Field trial Greytown
Entry	**Origin**	**UVPgt55**	**UVPgt57**	**UVPgt60**	**UVPgt61**	**UVPgt60**
W16/06	SGI	1	1	3-	;1	15MS
Sabie	SGI	3	3	3	3	20MS
Renoster	SGI	;1	1-	2	;1	60S
Koedoes	SGI	12+	0;	2++	3+	30MS
Umzumbi	SGI	;	1	3	;1	20MS
BSP12/13	SGI	3	;1	3	3	20MS
BSP13/08	SGI	3	0	3	3	30MR
BSP15/02	SGI	2, 3	;1	2+	;, 3	40MS
BSP13/18	SGI	2++	;1	2+	3	40MS
BSP16/01	SGI	3	3	2+3	3	40MS
BSP16/02	SGI	3+	;12+	2+3	3+	20MS
BSP16/03	SGI	3	;	3	3	40MRMS
BSP16/04	SGI	3	2++	3	3	50MS
BSP16/05	SGI	;12	;1, 1++	3+	3	70S
BSP16/06	SGI	3	12	2+	3	30MS
BSP16/07	SGI	3+	0;	3	3+	50MS
Kubetu_	SGI	;	;	2-	;	40MR
T08/21	SGI	2	0;	;1	3+	50MS
T13/01	SGI	3+	;n	4	3	70MS
T13/09	SGI	22+	2=	2++	3	80MSS
T13/10	SGI	;1	;	;1	;1	50RMR
T14/09	SGI	4	2=	3+	2++	90MSS
T15/03	SGI	2+	;	2-	2+	50MRMS
T15/08	SGI	2-	;1	2=	2=	50MRMS
T15/09	SGI	1	;1	1	1	60MRMS
T16/01	SGI	;1	0;	2	;1, 2	70MSS
T16/04	SGI	;1n	1	3+	;1	70MS
T16/07	SGI	;1	;	12+	;1	60MS
T16/09	SGI	;1	1-	2+	;1	40MS
T16/11	SGI	;1	;1	2+	;1	30MRMS
Wedzi_	SGI	3-	;1	22+	3-	30MR
Line 37-07*		3	3++	3+	3+	100S
Tobie*		;	3++	0;	;	na
Sr31 line*		;	2-	3++	;1	na
Morocco*		4	4	3++	4	na
McNair 701*		3+	3++	3++	3+	na

Mixed infection type scores: e.g. 2, 1p3 where most plants had a score of 2 while a single plant had a score of 3 or ;, 3 where an equal split is found.
nd: no data
na: not applicable
*Checks: Seedlings - Tobie (*Sr27, SrSatu*), Federation 4/Kavkaz (*Sr31*), Morocco and McNair 701 (*SrMcN*) (universal susceptibles)
Adult plants - Line 37-07 (susceptible)

Table 2.8: Percentage* South African varieties indicating seedling resistance to pathotypes of *Puccinia graminis* f. sp. *tritici*.

	UVPgt54	UVPgt55	UVPgt57	UVPgt60	UVPgt61	UVPgt62
2013		48.43	82.29	48.44	42.71	
2014		42.99	91.74	44.63	44.63	
2015	98.61	73.61	94.44	54.86	54.86	
2016		65.29	92.56	53.72	47.11	94.21
2017		54.31	92.24	50.00	44.83	

*All lines indicating resistance (Infection types < 3 and mixed reactions) were included

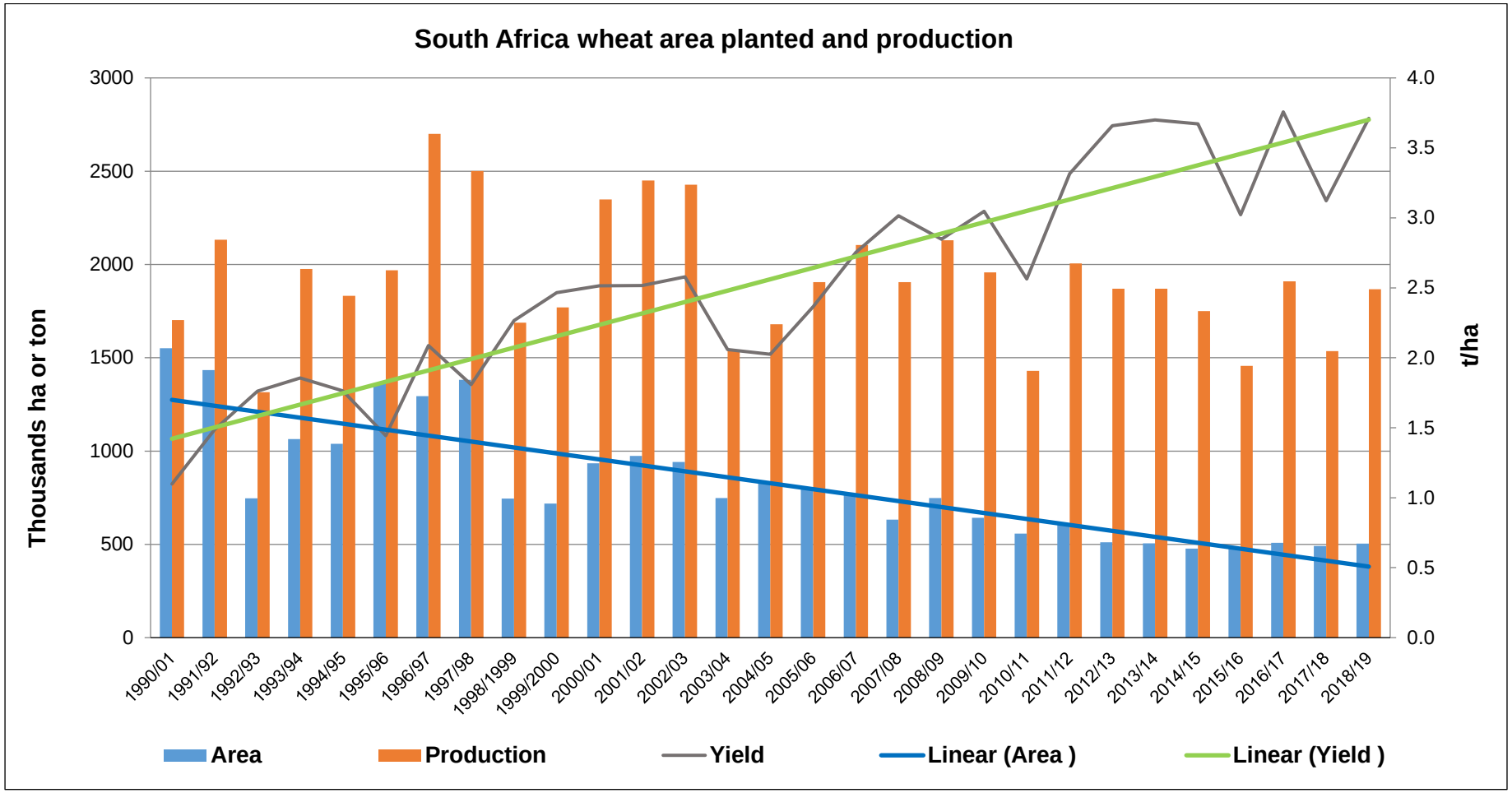

Figure 2.1: The production of wheat in South Africa from 1990/91 to 2018/19 (www.grainsa.co.za/pages/industry-reports/production-reports).

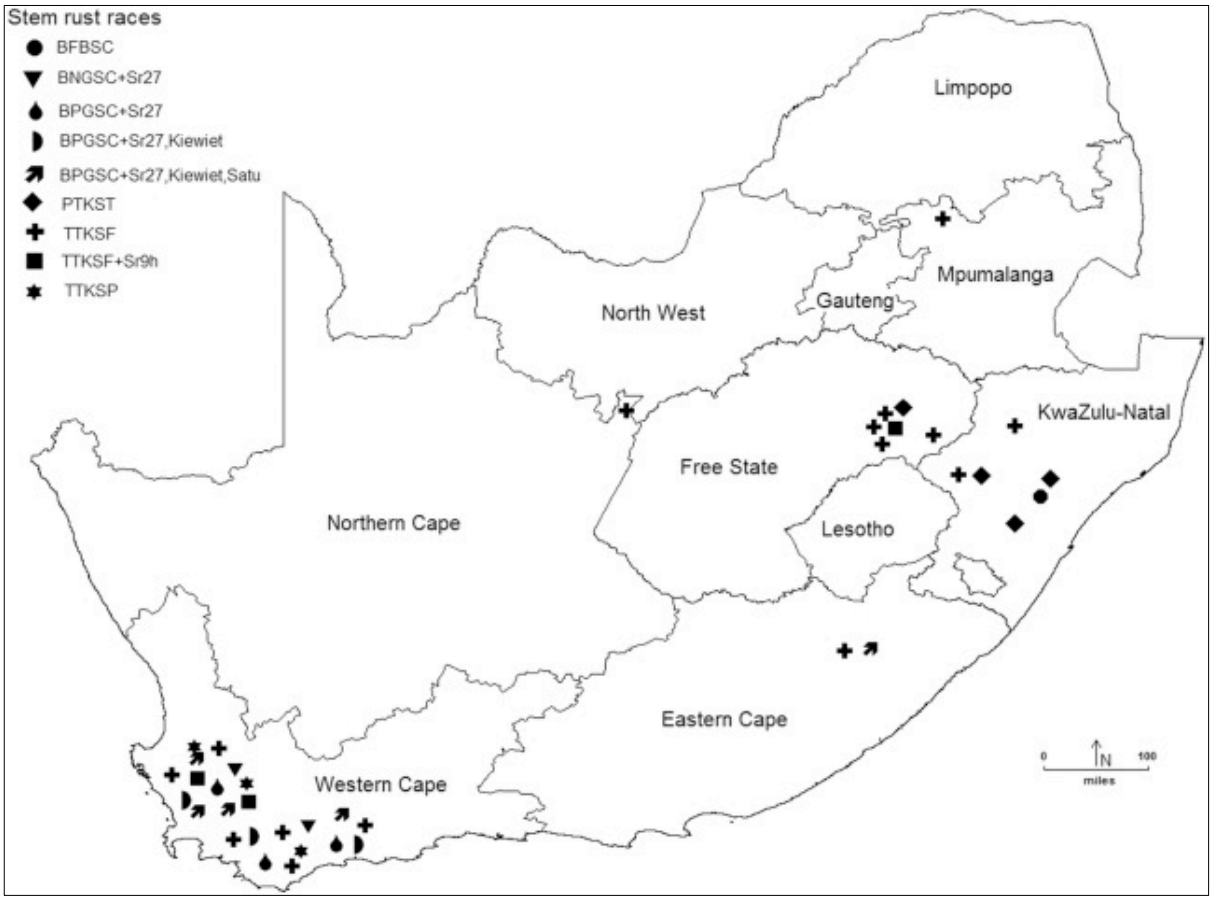

Figure 2.2: The distribution of *Puccinia graminis* f. sp. *tritici* pathotypes detected in the major wheat growing areas of South Africa during 2009 – 2013 (Terefe *et al*., 2016).

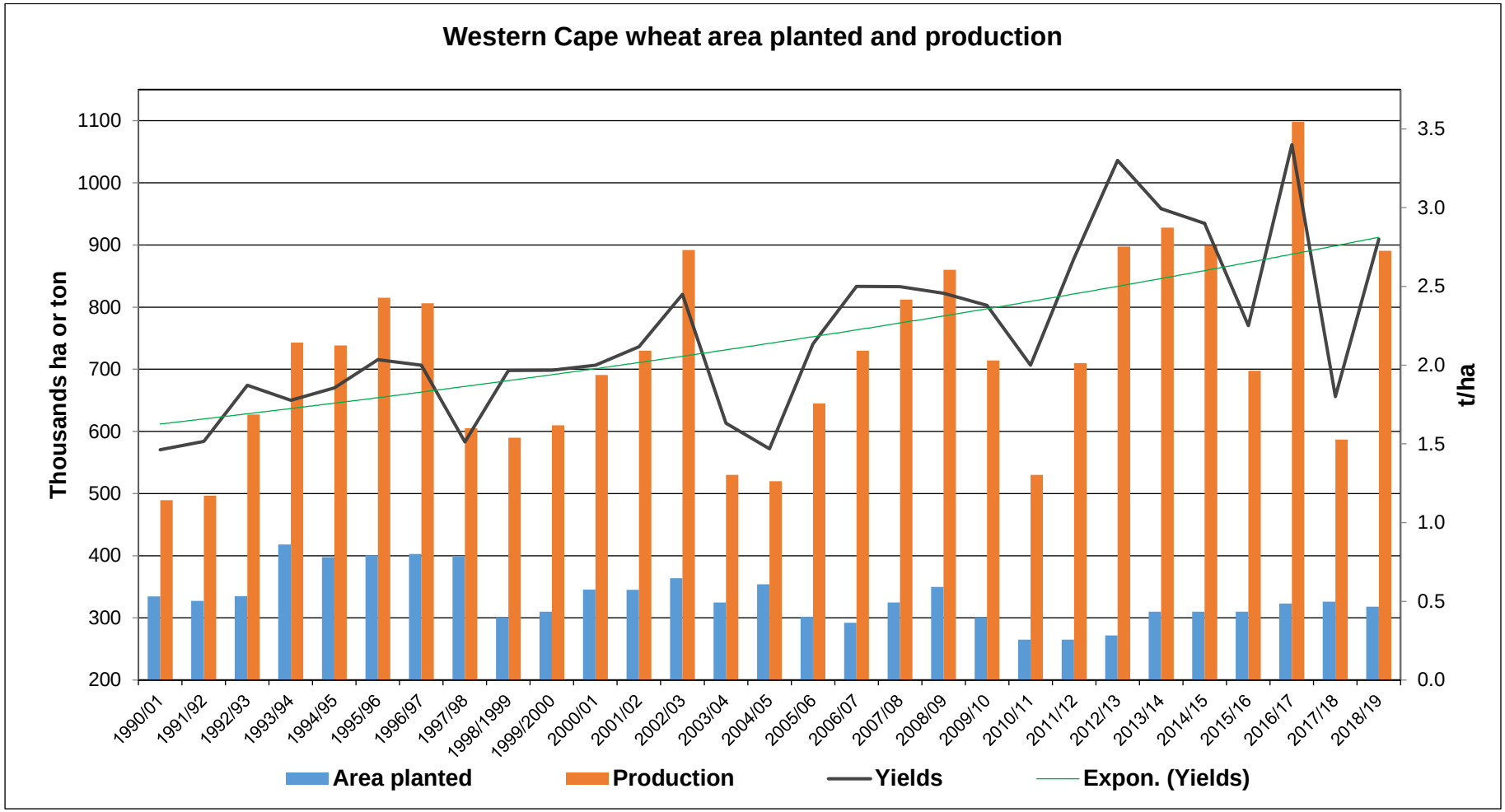

Figure 2.3: The production of wheat in the Western Cape from 1990/91 to 2018/19 (www.grainsa.co.za/pages/industry-reports/production-reports).

Figure 2.4: Major categories of *Puccinia graminis* f. sp. *tritici* infection types used to score wheat seedlings 14 days post inoculation where (from left to right) 0 = immune, ; = flecks, 1 = minute uredinia, 2 = small to medium sized uredinia, 3 = large uredinia sometimes encircled by slight chlorosis, 4 = large uredinia usually without any chlorosis.

Figure 2.5: Examples of adult plant reaction types to *Puccinia graminis* f. sp. *tritici* on wheat stems in the field (left to right): R=immune, R=resistant, MR=moderately resistant, MRMS=moderately resistant to moderately susceptible, S=susceptible.

Figure 2.6: Coefficient of Infection by *Puccinia graminis* f. sp. *tritici* on wheat stems in the field (left to right low to high): CI=5 (5S), CI=12 (40RMR), CI=32 (40MS), CI= 60 (60S).

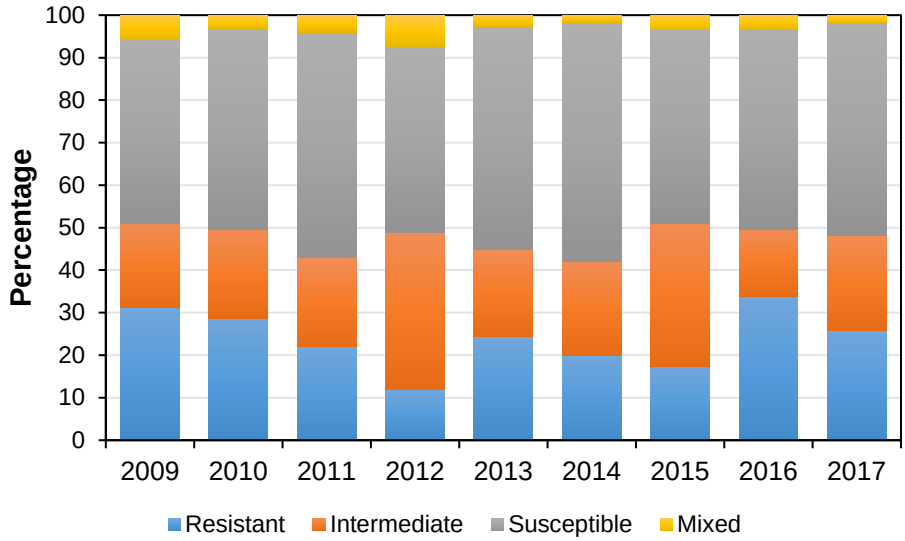

Figure 2.7: Summary of seedling resistance from 2009 to 2017 as a percentage to *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 in 2009 and UVPgt60 since 2010 in South African wheat varieties.

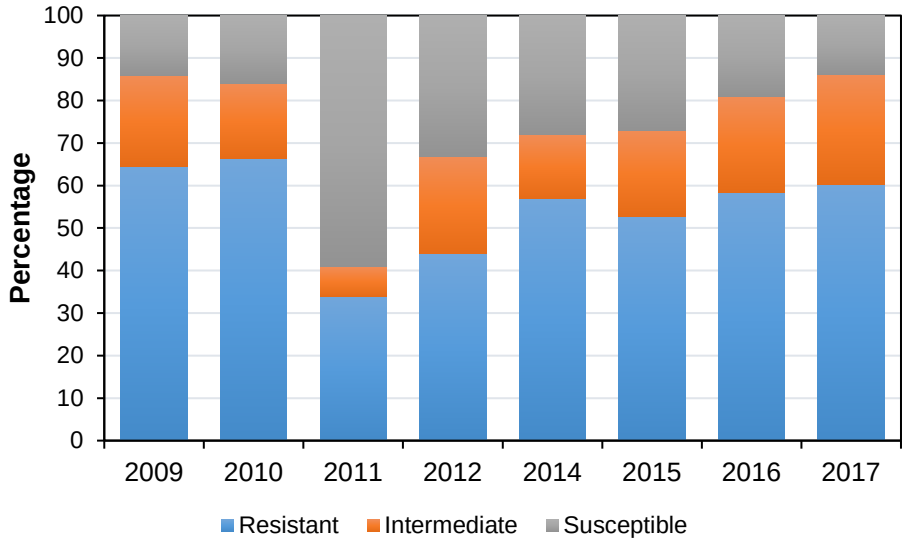

Figure 2.8: Summary of adult plant resistance from 2009 to 2017 as a percentage to *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 in 2009 and 2010 and UVPgt60 since 2011 in South African wheat varieties.

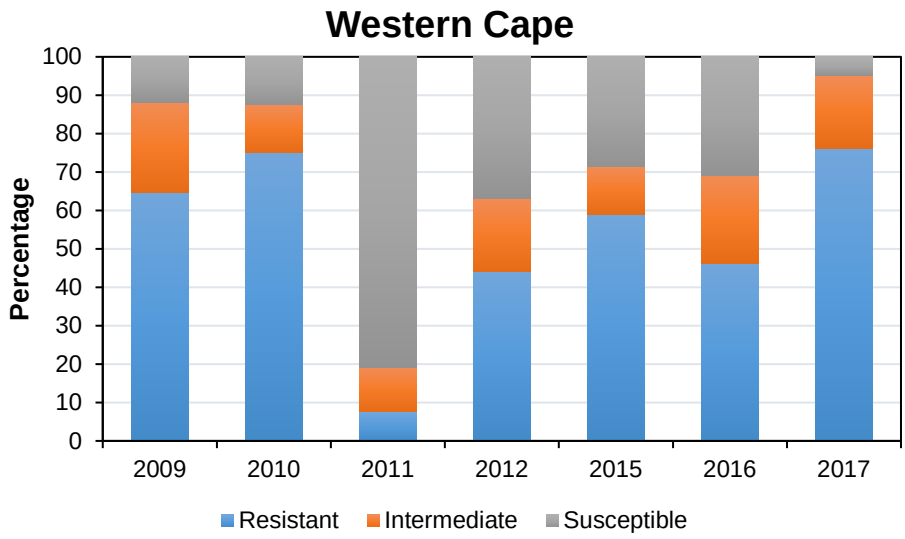

Figure 2.9: Summary of adult plant resistance from 2009 to 2017 to *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 in 2009 and 2010 and UVPgt60 since 2011 in South African wheat varieties developed for the Western Cape production area.

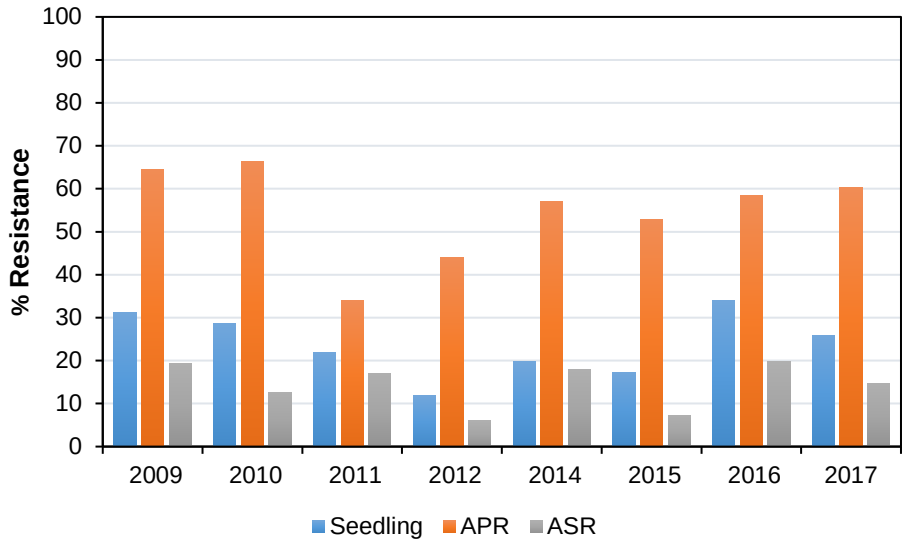

Figure 2.10: Summary of the frequency of occurrence of seedling, adult plant (APR) and all stage resistance (ASR) from 2009 to 2017 in South African wheat varieties. In 2009 *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 (seedling and adult plants), 2010 UVPgt60 (seedlings) and UVPgt59 (adult plants) and since 2011 UVPgt60 (seedling and adult plants) were used.

Chapter 3: Development of a greenhouse screening method for adult plant response to stem rust of wheat

INTRODUCTION

Stem rust of wheat (*Triticum aestivum* L.), caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., (*Pgt*) is regarded as one of the most damaging diseases of wheat because of its pathogenic variability, adaptation to long-distance wind dispersal, epidemic potential and ability to cause significant losses (Dean *et al.*, 2012). The importance of wheat stem rust was re-emphasized by the detection of African race “Ug99” (North American race code TTKSK) in Uganda (Pretorius *et al*., 2000b). This discovery, in particular the occurrence of virulence for the commonly used *Sr31* resistance gene, has since initiated a global effort to combat the disease (McIntosh and Pretorius, 2011). At present 13 variants within the Ug99 race group are known, differing in virulence for *Sr9h*, *Sr21*, *Sr24*, *Sr30*, *Sr31, Sr36* and *SrTmp* (Singh *et al*., 2011, 2015, Pretorius *et al*., 2012a, Patpour *et al*., 2016, Fetch *et al*., 2016). Distribution of Ug99 variants has been confirmed in Egypt, Eritrea, Ethiopia, Iran, Kenya, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Uganda, Yemen, and Zimbabwe (Singh *et al*., 2008, Nazari *et al*., 2009, Visser *et al*., 2011, Pretorius *et al*., 2012b, Patpour *et al*., 2016, Terefe *et al*., 2019, www.rusttracker.org [accessed November 20, 2019]).

Although fungicides can be used to control wheat stem rust (Wanyera *et al*., 2009), chemical protection is expensive, requires expertise, the availability of spraying equipment and a supply of registered products. Thus, the deployment of resistant cultivars is widely accepted as the best way to reduce inoculum levels and to prevent or control the disease, especially for resource poor farmers. Since rust pathogens have the ability to adapt and acquire virulence for resistance genes, the ongoing identification, characterization and utilization of sources of resistance are needed.

Resistance to stem rust is broadly classified as all stage resistance, which can be detected in wheat seedlings and all subsequent growth stages, and adult plant resistance (APR), which is most effective in mature plants in the field (Lagudah,

2011, Ellis *et al*., 2014). However, the efficiency of stem rust phenotyping can be improved by scoring adult plant responses in a greenhouse where the racial identity of inoculum and uniformity of application can be controlled. The objective of this study was to develop a dependable screening system for assessing APR for stem rust in the greenhouse. Procedures were optimized using sets of wheat lines differing in their response to stem rust.

MATERIAL AND METHODS

Experiment 1

The feasibility of infecting adult wheat plants with *Pgt* was investigated on cultivars Inia 66 (*Sr2*), Hartog (*Sr2*), Kariega (susceptible) and Morocco (susceptible). Wheat plants were grown in sterilized soil in tapered plastic cones (4 x 4 x 10 cm) (three plants per entry per cone) at 18-25°C in a greenhouse. Cones were positioned in a 14 x 7 unit tray, supported by a corresponding, raised grid of nylon line to prevent plants from lodging (Pretorius *et al*., 2007). After emergence plants were fertilized twice with Multifeed® water-soluble fertilizer (19:8:16) NPK plus micronutrients, concentration 2.5 g/L water), followed by a water suspension of Wonder® 3:2:1, a slow nitrogen release granular fertilizer, at 7-day intervals for the duration of the trial.

Plants were inoculated with pathotype UVPgt59 (TTKSP, avirulent for *Sr9h, 26, 27, 31, 36, Kiewiet, Satu, Tmp* and virulent for *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 24, 30, 38, McN*) 51 days after planting when the majority were flowering (Zadoks growth stage 63). Three inoculation methods, two incubation environments, and two moisture duration periods were tested. In the first inoculation method, fresh urediniospores were suspended in light mineral oil (5 mg/ml) in gelatin capsules (Fig 3.1) and sprayed onto plants using the system primarily developed for applying rust spores to seedlings (Browder, 1971). Secondly, spores were suspended in distilled water (2.5 mg/ml) and Tween 20® (0.03% v/v) and applied to stems using a compressed air sprayer (Fig 3.2), similar to the procedures for inoculating adult wheat plants with stripe rust (Pretorius *et al*., 2007). Thirdly, dry spores (0.008 g) were applied for 3 min to horizontally positioned plants in a settling

tower (Fig 3.3) (Negussie and Pretorius, 2005). The same source of freshly collected urediniospores of UVPgt59, increased in bulk on LCSr24Ag seedlings, was used in the respective experiments.

Plants from all entries and inoculation methods were incubated either in the dark in a condensation dew chamber (Fig 3.4 A) or in natural light/darkness in a plastic chamber (Fig 3.4 B) in a greenhouse. Treatments were randomized within each chamber. The condensation chamber, manufactured from metal sheeting and measuring 180 cm wide x 120 cm high x 100 cm deep, functioned in an air-conditioned room at 20°C. Dew was formed by vapor released from two water baths set at 40°C and recessed in the bottom of the chamber. The plastic chamber measured 100 cm x 85 cm x 60 cm and consisted of an aluminum frame covered with 200 µm greenhouse sheeting (Evadek [green tint], Gundle® API Plastics, Springs, South Africa). The plastic-covered frame was placed in a tray filled with water to a depth of approximately 5 cm. Inside the frame, plants were placed on a metal grid raised above the water line. Plants were incubated for either 12 h or 24 h. The temperature ranges in the greenhouse cubicle housing the plastic chamber and condensation dew chamber were 20-28°C and 20-24°C, respectively. Each treatment was replicated three times, with three cones per replicate.

When the dew cycles ended, plants were dried at 20°C before placing them in a greenhouse set to an 18-25°C night/day schedule. Stem rust infection reaction types (resistant R, moderately resistant MR, moderately susceptible MS, and susceptible S) (Stubbs *et al*., 1986) were recorded and the total number of pustules on a 5 cm stem section of the second last internode was counted 14 days post-inoculation.

Experiment 2

The water/spore suspension inoculation method was refined in a second experiment which was conducted twice. The same entries as above and an experimental line (ZA2006-28 EXP.3) with *Sr24* and *Sr31* were used (Pretorius *et al*., 2012a). Growth conditions before and after inoculation were as described above. Using a spray-gun and compressor, 100 ml of each of three spore

concentrations (0.67, 1.25 and 2.5 mg/ml) of pathotype UVPgt59 were applied as uniformly as possible to approximately ~100 adult plants per concentration. Plants were incubated as described earlier in either a dark dew chamber or in plastic tents in a greenhouse for 24 h. A three disc mist maker was added to one of the plastic tents. A CR10X data logger (Campbell Scientific, Inc. www.campbellsci.com, Somerset West, South Africa) monitored temperature, humidity and leaf wetness through HMP45C probes and a dielectric leaf surface wetness sensor (Decagon Devices, Inc. www.decagon.com, Stellenbosch, South Africa). Stem rust incidence (percentage infected plants) and infection response types were determined 14 days after inoculation. The coefficient of infection (CI), where severity according the modified Cobb scale is multiplied with a constant for host infection reaction (R = 0.2, MR = 0.4, MRMS = 0.6, MS = 0.8 and S = 1, Roelfs *et al*., 1992), was calculated.

Data analysis

Analysis of variance was done with NCSS Statistical Software System (Hintze, 2007). A completely randomised design was used for data analysis.

RESULTS

Experiment 1

The adult plant infection reaction type ranged from S for Morocco and Kariega to MRMS for Hartog, and MS for Inia 66 (Fig 3.5). In successful infections the first pustules on susceptible entries were visible 6 days after inoculation (Fig 3.6). The number of pustules on a 5 cm stem section was significantly ($P<0.05$) influenced by inoculation and incubation methods used. Very little stem rust developed on plants incubated in the condensation dew chamber in the first experiment (Fig 3.7 A). In this chamber only trace amounts of rust were observed on plants inoculated with dry spores or with a spore/water suspension. Some stem rust occurred on plants inoculated with oil, with those incubated for 24 h showing marginally more pustules than the 12 h dew period (Fig 3.7 A). High levels of stem rust developed on Kariega and Morocco plants inoculated with spores suspended in water, and incubated in the plastic chamber for 24 h (Fig 3.7 B). In this treatment the rust levels on stems of the more resistant Hartog and Inia 66 were proportionally lower than on the susceptible entries. Some stem rust occurred in the dry spore and spore/oil

treatments with indications of higher infection levels after a 24 h incubation period (Fig 3.7 B).

Experiment 2

In the second experiment cultivar reaction types were similar to those reported above. The *Sr24+31* line showed R to MR infection reaction types to pathotype UVPgt59 (Fig 3.5). Results of the two repeats were not significantly ($P<0.05$) different and data were pooled. The percentage of infected plants was significantly ($P<0.05$) influenced by genotype and incubation method used. Stem rust incidence on plants incubated in the condensation chamber was low and inconsistent (Fig 3.8 A). In contrast, a high incidence (65-100%) was observed on plants incubated in the plastic tent without the mist maker (Fig 3.8 B). However, in the plastic tent where extra moisture was added by the mist maker, the percentage stem rust infected plants decreased, varying between 20% and a maximum of 85% (Fig 3.8 C). High temperatures were reached inside the chamber in the greenhouse with a maximum of 38°C shortly after incubation commenced. Night temperatures were stable at 19-20°C during 22:40 to 06:40, again rising to 38°C when the incubation was terminated at 13:00 (Fig 3.9 B). Condensation chamber temperatures ranged between 21°C and 24°C (Fig 3.9 A). Here, relative humidity was 42% at the onset of the experiment but increased to 98% 1 h later, which was maintained for the duration of the cycle (Fig 3.10 A). Relative humidity in the greenhouse chamber started at 35%, rising to 75% after 1 h, reached a maximum of 97 % at 02:00 before decreasing to 75% when the plants were removed (Fig 3.10 B). The quantification of leaf wetness showed a slower onset and lower levels of moisture on plants in the plastic chamber (Fig 3.11 B) compared to the condensation chamber Fig 3.11 A). Spore concentration did not significantly ($P<0.05$) influence the percentage of plants showing stem rust symptoms. However, pustule size on the susceptible entries was restricted in the 2.5 mg/ml treatment. Severity values as high as 80% were observed on Morocco plants. CI data supported the expected reaction type range from resistant to highly susceptible (Fig 3.12).

DISCUSSION

APR to the rust pathogens is a priority in most programs breeding for disease

resistance in wheat (Lagudah, 2011, Agenbag *et al*., 2012, Singh *et al*., 2011, 2015, Njau *et al*., 2013, Tsilo *et al*., 2014, Yu *et al*., 2014). Accurate assessment of partial resistance, which by definition includes many APR phenotypes, is essential for developing varieties carrying this type of resistance (Ayliffe *et al*., 2013). Lowe *et al*. (2011) mentioned that a definitive disease phenotype, whether quantified macroscopically or microscopically, is the starting point for understanding partial resistance in context with its molecular mechanisms, eventually leading to gene cloning and application in breeding. Likewise, Ellis *et al*. (2014) stated that a detailed characterization and understanding of APR is required to advise rust resistance breeding. Screening for APR classically involves assessment of rust infection in field plots that could be costly, time consuming, and its expression dependent on unwanted biotic and abiotic stress factors (Hickey *et al*., 2012). Thus, a suitable greenhouse screening method presents an additional prospect for studying stem rust in adult plants provided that such a system is reliable, accurate and efficient in terms of high-throughput capacity.

The concept of improving and accelerating adult plant rust phenotyping in a controlled environment was presented by Pretorius *et al*. (2000a) who showed that APR to leaf rust and stripe rust was clearly expressed in flag leaves of plants grown under continuous light and inoculated 28 days after sowing. When the same approach was followed in a mapping study, not all stripe rust resistance QTL were expressed in a Kariega x Avocet S DH population (Ramburan *et al*., 2004). However, Pretorius *et al*. (2007) showed that improved APR results with stripe rust were obtained when more vigorous plants were grown in a greenhouse as opposed to the mini-adults raised under constant light in a growth chamber. Hickey *et al*. (2012) found that APR to stripe rust could be reliably detected from leaf reactions of wheat varieties inoculated at the stem elongation stage three weeks after planting. When plants were grown under continuous light both Ramburan *et al*. (2004) and Hickey *et al*. (2012) indicated that younger leaves were more susceptible than older leaves suggesting an environmental effect on the expression of stripe rust resistance in young plant tissues. Working with leaf rust Singh (1992) reported that low infection types were best recognized in older leaves, which supports the approach of phenotyping sturdy plants at an appropriate growth stage.

Less research has been done on greenhouse assessment of stem rust-infected adult wheat plants. Sunderwirth and Roelfs (1980) determined anthesis as the best growth stage for reliable greenhouse observations of *Sr2* expression. They found that the number and size of uredinia of *Pgt* were significantly reduced on adult plants containing *Sr2*. Typically, susceptible type lesions on *Sr2* plants were situated above the nodes. Eaton *et al*. (1984) determined stem rust infection at two mature growth stages in a greenhouse but concluded that the prediction of field infection levels using greenhouse variables was not possible.

Although a reliable greenhouse method was developed in the present study, marked differences occurred in the successful outcome of inoculation methods combined with incubation environments. In general the lowest infection levels were achieved in the condensation dew chamber which maintains free moisture, but without run-off, on plant surfaces. This facility, in which plants are incubated in darkness, has been successfully used for consistent infection of seedlings in several rust pathosystems, including wheat stem rust, for over 25 years. Although temperature inside the chamber was within the boundaries of 15-24°C for optimal germination of *Pgt* urediniospores (Sharp *et al*., 1958), low levels of stem infection occurred. This is particularly true for plants either inoculated with dry spores or with a water-spore suspension containing a wetting agent. Similarly, low infection levels under controlled conditions were experienced by Rowell (1958) who found that even with 98-100% and 50-60% germination and appressorium formation rates, respectively, only 15 to 25% successful infections occurred.

The best and most dependable results were obtained with adult plants incubated in the plastic chamber. The obvious differences between the two incubation environments were moisture and light, with heavy dew and no light prevailing in the condensation chamber, as opposed to less moisture and several hours of natural, yet filtered daylight in the plastic chamber. Rowell (1984) mentioned that many appressoria remain quiescent when plants are left in dark, moist conditions but will develop penetration structures if the temperature is raised to 30°C, either in darkness or in light. Staples and Macko (1984) stated that germination of urediniospores of *Pgt* is inhibited by continuous irradiation, even before the germ

tubes emerge. Burrage (1970) reported that light inhibited germination of urediniospores on wheat leaves but several studies have pointed out that *Pgt* is rather unusual since substomatal vesicle formation requires exposure to light (Rowell, 1958, Sharp *et al*., 1958, Yirgou and Caldwell, 1968). Given viable urediniospores and their effective deposition on plants, it appears that prolonged dew formation on stems within the required temperature window is not necessarily the only requirement for successful infection of adult plants. From our results the initial stem wetness provided by the water-based inoculum spray, together with the water tray in the bottom of the plastic chamber, provided enough humidity for spore germination. According to our side-by-side comparison of dew chambers, an incubation period of 24 h, including a natural light and dark phase, and/or less free moisture within a plastic chamber, were conducive to the stem infection process.

The possibility of closed stomata as a physical factor in reduced rust infection was regarded as unlikely by Rowell (1984), but elevated respiratory CO_2 levels in the dark may play a role in poor infection. In addition, a variety of organic and inorganic substances in water may inhibit germination of rust spores (Rowell, 1984). Based on our experience of successful seedling infection using this system, it is unlikely that water quality was responsible for the poor infection obtained in the dew chamber. Moisture is deposited on plant surfaces through condensation when vapor produced by the heated water baths, presumably without impurities, is cooled by the chamber walls. Furthermore, only water purified through reverse osmosis and sterilization was used in water-spore suspensions and successful infection in the plastic chamber ruled out water as a restraining factor.

In our study it was possible that spores germinated in the dark chamber but that the adult plant infection process could not be completed due to an unfavorable light-temperature-moisture interaction. Although our investigation did not resolve which factors were critical for stem infections, it provided development of a successful assay for mature plants. In the present study plant height (~ 65 cm), flag leaf length (~ 20 cm) and spike length (~ 6 cm, excluding awns) confirmed more robust plant growth (actual data not shown), thus avoiding the sensitive tissues of light-accelerated mini-adult plants. The quick maturation of flag leaves encountered

previously (Pretorius *et al*., 2007) was not seen in the current work and we concluded that plant development was suitable for obtaining quality stem rust data. Using 100 ml inoculum for 30 entries provided adequate stem coverage and a concentration of 1.25 mg/ml spores suspended in water and Tween 20 was sufficient. The high density of pustules on the susceptible control Morocco suggested that lower concentrations, allowing larger uredinium growth, should be considered. Inoculum pressure through polycyclic uredinium production in the field versus a single infection phase in the greenhouse might explain most variation observed in severity rather than reaction type. Nevertheless, these data demonstrated that a single infection cycle in the greenhouse can either supplement, or, depending on the objective, substitute for field scoring.

The controlled status of the greenhouse method resulted in clear observations of when signs of *Pgt* infections commenced. The first signs of uredinia were visible 6 days after inoculation and it would thus be possible to use this technique for assessment of macroscopic components of APR such as latent period, pustule size and pustule density. The stems of greenhouse-grown material were uniformly infected by rust, confirming that a reliable level of infection can be achieved using the conditions as described. In terms of microscopic resistance components the procedure is particularly adapted to the method described by Ayliffe *et al*. (2013) for quantification of fungal biomass. While their study used seedlings for wheat stem rust and flag leaves from field plots for leaf rust and stripe rust, the wheat germ agglutinin chitin (WAC) assay can equally well be applied to greenhouse-grown leaf sheath tissue to quantify *Pgt* in adult plants.

In conclusion, this study describes a functional system for controlled infection of adult wheat plants with *Pgt* for phenotyping fixed lines and populations, and for further application in germplasm characterization, inheritance studies, resistance mapping and refined host-pathogen interaction studies. It also lays a foundation for improving moisture, temperature and light requirements for application in epidemiological studies. Should the interaction between resistance genes in adult plants be a priority of investigation, the currently described system will be more supportive. Lower CI’s were observed on varieties such as Inia 66 and Hartog which

contain the APR gene *Sr2,* providing evidence that infection of adult plants in a greenhouse will be an additional instrument for stem rust resistance assessments.

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Figure 3.1: Inoculation of wheat plants with urediniospores of pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici* suspended in light mineral oil in a gelatin capsule.

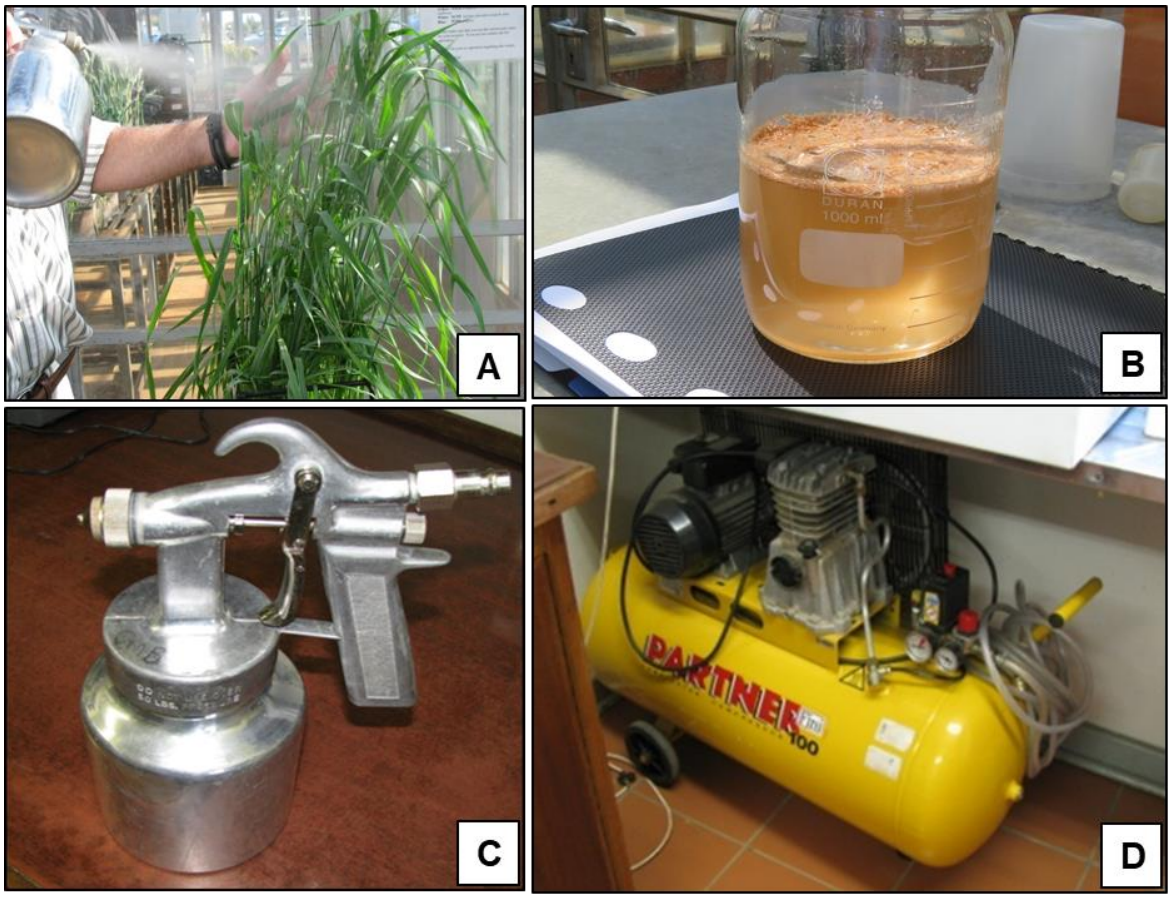

Figure 3.2: Inoculation of wheat plants (A) with urediniospores of pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici* suspended in distilled water and Tween 20 (B) using an airgun (C) and compressor (D).

Figure 3.3: Settling tower used for inoculation of wheat plants horizontally positioned with dry urediniospores of pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici*.

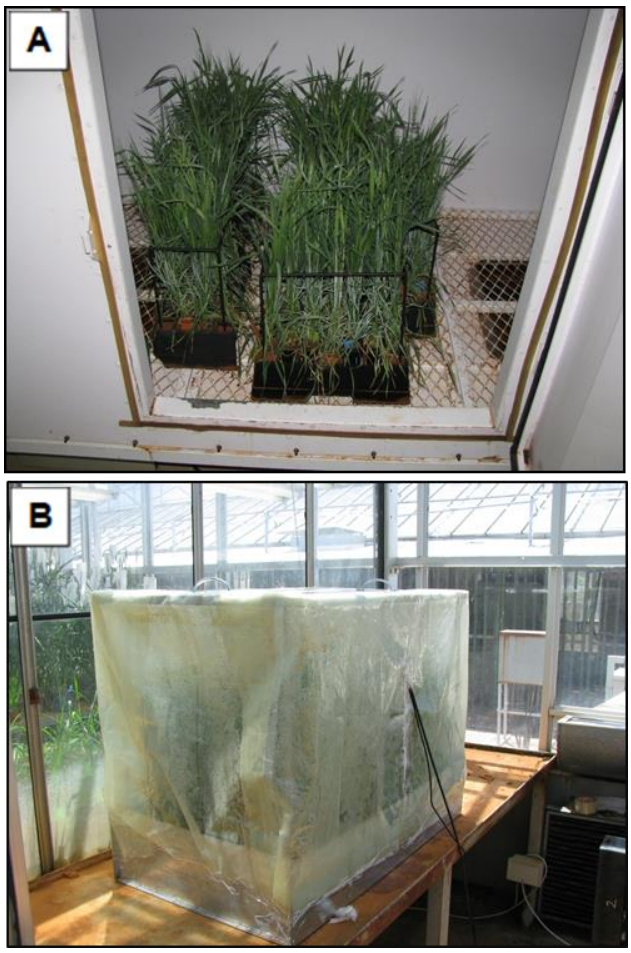

Figure 3.4: Incubation of wheat plants inoculated with urediniospores of pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici* in (A) the dark in a condensation chamber or (B) in the greenhouse in a plastic chamber.

Figure 3.5: Adult plant infection reaction types of pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici* on stems of the wheat varieties ZA2006-28 EXP.3, Hartog, Inia 66, Kariega and Morocco (left to right): R= resistant, MRMS=moderately resistant to moderately susceptible, MS=moderately susceptible, S=susceptible.

Figure 3.6: The first visible stem rust symptoms on stems of the cultivar Kariega six days after inoculation with pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici*.

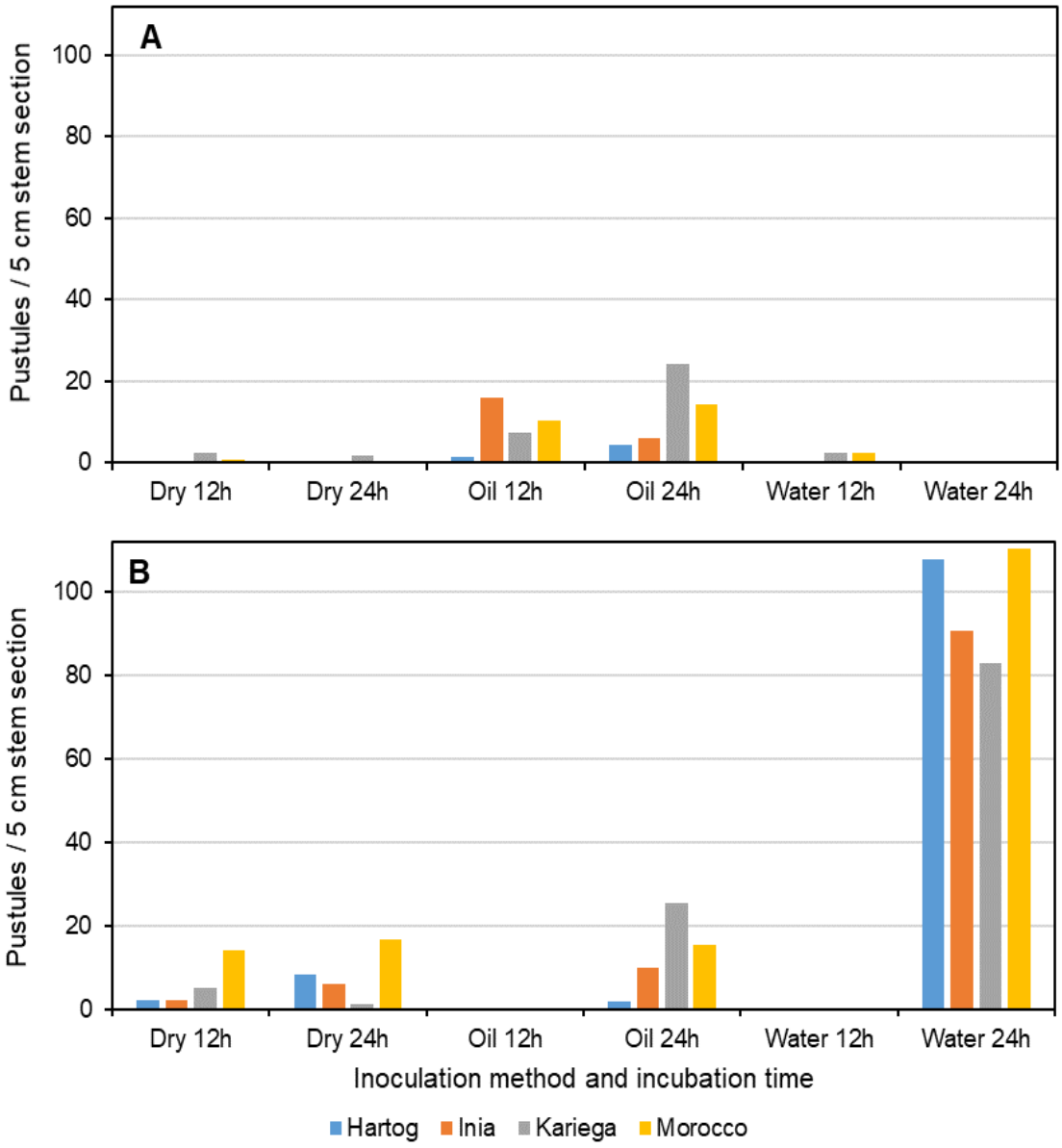

Figure 3.7: Mean number of stem rust pustules counted 14 days after inoculation on a 5 cm stem section of adult plants of four cultivars inoculated with *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 and incubated in the dark in a condensation dew chamber (A) and in natural light/darkness in a plastic chamber in a greenhouse (B). Least significant differences (LSD) among lines for number of pustules per 5 cm stem section were calculated as 4.2 in a condensation dew chamber (A) and as 8.0 in a plastic chamber in a greenhouse (B) ($P \leq 0.05$ ANOVA).

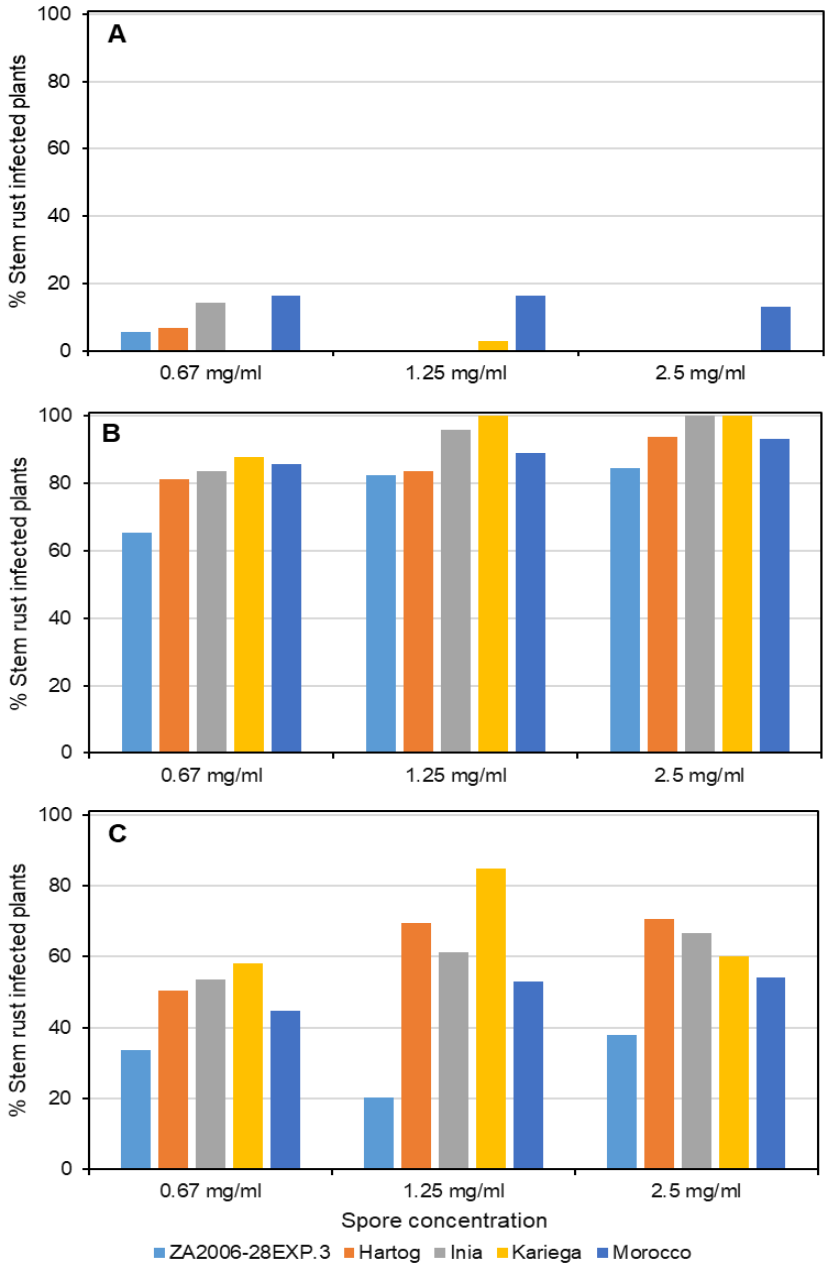

Figure 3.8: Stem rust incidence (percentage plants infected) 14 days after inoculation on adult plants of five wheat cultivars inoculated with *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 and incubated in the dark in a condensation dew chamber (A) and in natural light/darkness in two plastic chambers in a greenhouse (B) without added moisture and (C) with added moisture. Least significant differences (LSD) among lines for percentage plants infected were calculated as 6.5 for the condensation dew chamber (A) and for the plastic chambers in the greenhouse as 21.6 for (B) and as 11.6 for (C) ($P \leq 0.05$ ANOVA).

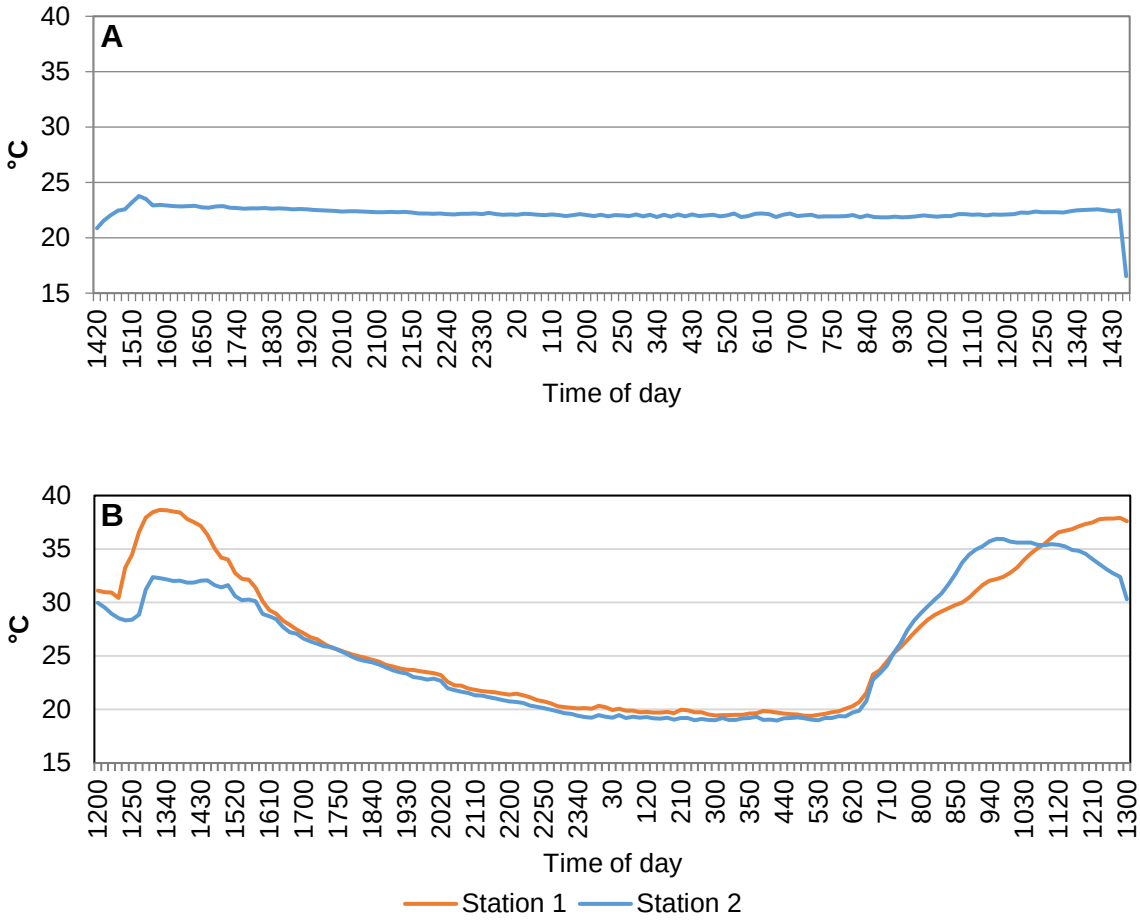

Figure 3.9: Temperatures measured in a condensation dew chamber (A) and in natural light/darkness in two plastic chambers in a greenhouse (B). Station 1 represent the chamber with added moisture and station 2 the chamber without added moisture.

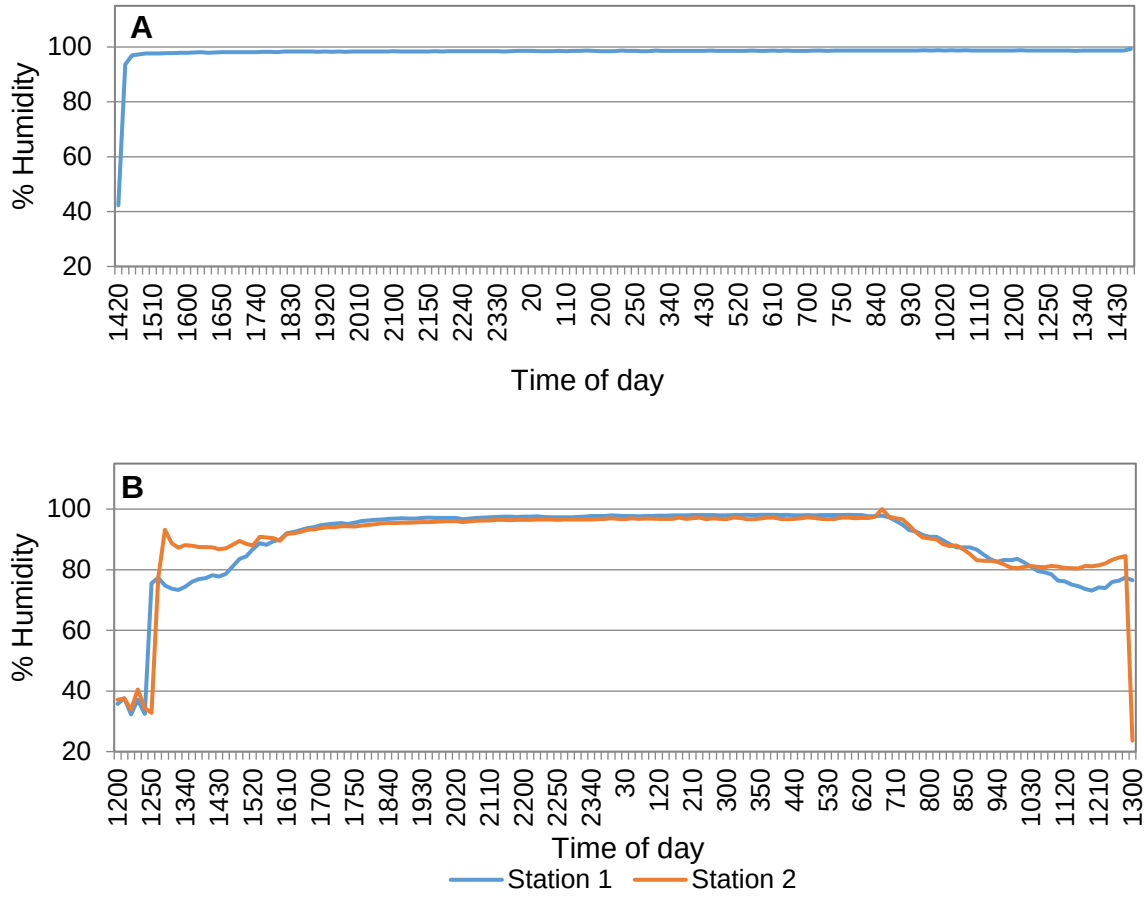

Figure 3.10: Relative humidity measured in a condensation dew chamber (A) and in natural light/darkness in two plastic chambers in a greenhouse (B). Station 1 represent chamber with added moisture and station 2 the chamber without added moisture.

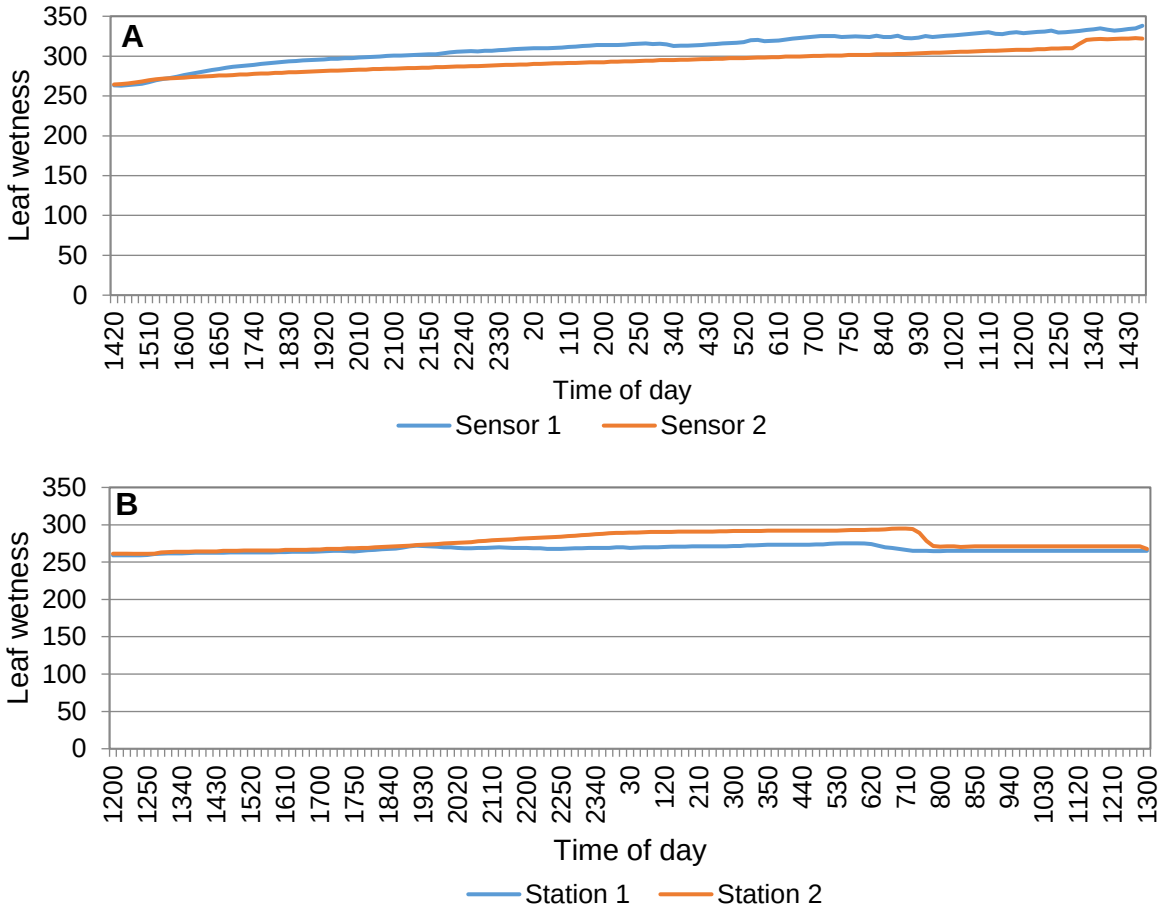

Figure 3.11: Leaf wetness measured with two sensors in a condensation dew chamber (A) and in natural light/darkness in two plastic chambers in a greenhouse (B). Station 1 represent chamber with added moisture and station 2 the chamber without added moisture.

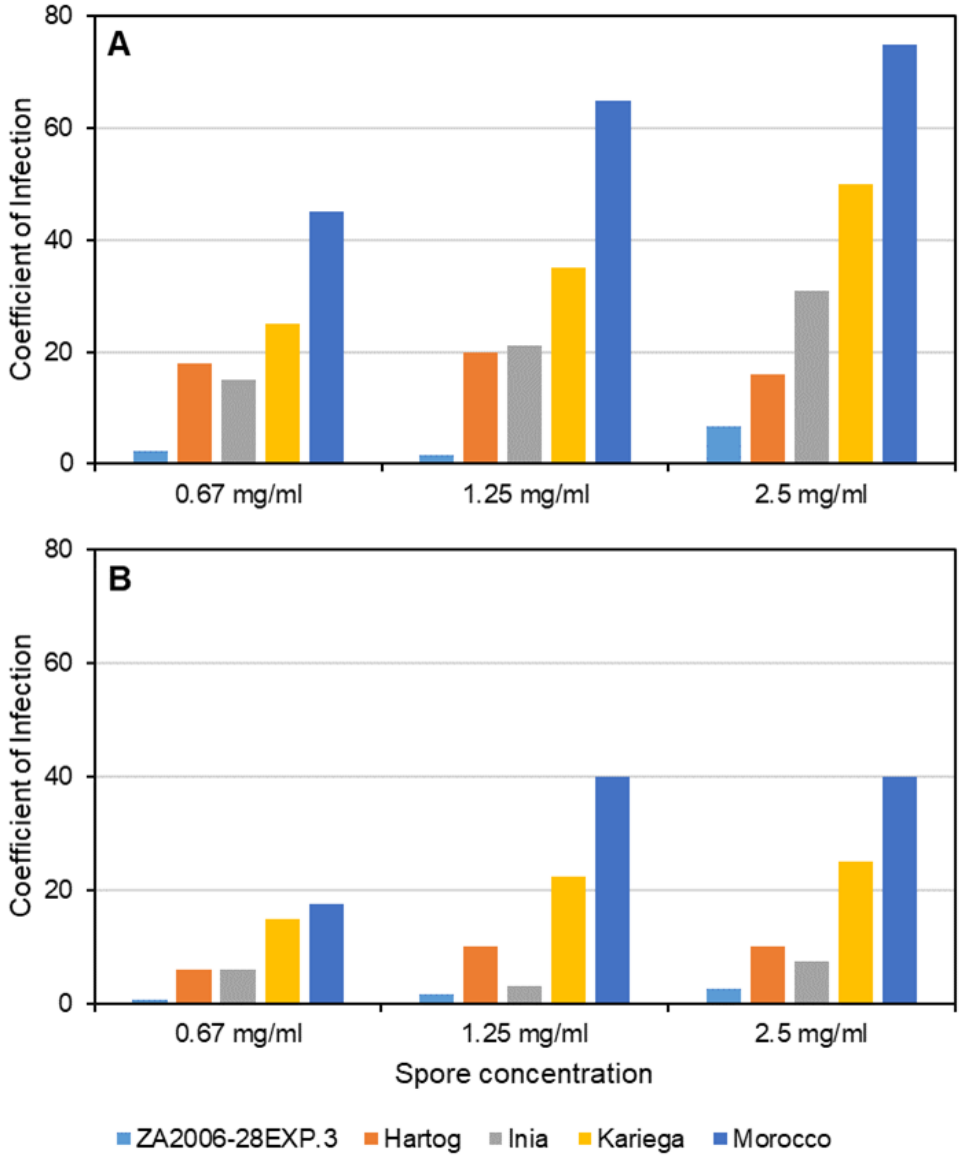

Figure 3.12: Comparison of coefficient of infection of three different spore concentrations of *P. graminis* f. sp. *tritici* pathotype UVPgt59 on five cultivars calculated 14 days after inoculation. Plants were incubated in natural light/darkness in a plastic chamber in a greenhouse without added moisture (A) and with added moisture (B). Least significant differences (LSD) among lines for coefficient of infection in the greenhouse were calculated as 19.0 for (A) the plastic chamber without added moisture and as 12.2 for (B) the plastic chamber with added moisture ($P \leq 0.05$ ANOVA).

Chapter 4: Validation of a greenhouse screening method for adult plant response to stem rust in wheat

INTRODUCTION

Pathotypes belonging to the Ug99 lineage of the stem rust fungus *Puccinia graminis* f. sp. *tritici* (*Pgt*) is a threat to wheat (*Triticum aestivum* L.) production worldwide. When susceptible cultivars are grown in rust prone areas, complete yield losses can occur during epidemic outbreaks (Saari and Prescott, 1985, Dean *et al*., 2012). Genetic resistance to stem rust is the most economical and environmental friendly control measure. Therefore, evaluation of wheat germplasm to detect resistance is an ongoing practice by breeders and plant pathologists which necessitates continuous and reliable assessment of stem rust response.

To improve stem rust phenotyping, a greenhouse screening method to characterise adult plant resistance (APR) was developed (Chapter 3; Bender *et al*., 2016). Benefits of the method include control over the racial identity of inoculum, uniformity of application thereof, and not only reliable, but also relatively rapid results. The question remains whether greenhouse data accurately reflect field data. A verified greenhouse method for stem rust screening could be advantageous in time, accuracy and cost when compared to field trials. Validation should demonstrate that the newly described technique presents a practical and dependable screening method for assessing stem rust APR in wheat.

The objective of this study was to verify the greenhouse inoculation method using South African wheat cultivars, selected entries from the Elite Spring Wheat Yield Trial (ESWYT) provided by the International Maize and Wheat Improvement Center, Mexico (CIMMYT), and a doubled haploid population known to carry *Sr5*, *Sr26* and *Lr34/Yr18/Sr57* (Prins *et al*., 2011). The technique was furthermore corroborated with the urediniospore spore carrier Novec™ 7100 (3M™), an engineered fluid that is more effective for virulence phenotyping with spray inoculation according to Sørensen *et al*. (2016).

MATERIAL AND METHODS

Wheat genotypes

Three different wheat germplasm groups were used for validating the developed greenhouse method. The first group included 20 South African spring wheat cultivars varying in APR responses. The 30th ESWYT (Appendix A) included as the second group was evaluated in the greenhouse and in field trials in Kenya and Greytown, South Africa (SA). Twenty-four lines, mostly expressing APR, were selected for greenhouse screening from the 30th ESWYT (R. Singh, personal communication). PBW343 (a selection made at Punjab Agricultural University, Ludhiana, Punjab, India, from lines developed at CIMMYT, Mexico, a mega cultivar that carries *Sr31* (accessed November 2019 http://wheatpedigree.net /sort/show/79183) was one of the entries of the 30th ESWYT. The universally susceptible wheat cultivar Morocco was included as a control in all trials, whereas the stem rust-susceptible entry Line 37-07 was added as a further control. Line 37-07 (pedigree: Kasyob/Genaro-81//Cham4) was selected from entry 37 of the 2nd International Stem Rust Trap Nursery (ISRTN07) based on its susceptibility to stem rust in field trials in SA. The last group consisted of a Kariega x Avocet S doubled haploid (DH) mapping population of 254 entries where the parental lines Kariega and Avocet S served as controls.

For adult plant trials seed were planted in sterilized soil in tapered plastic cones (4 x 4 x 10 cm) (three plants per entry per cone) in a 14 x 7 unit tray. All plants were grown in a disease-free greenhouse at 18-25°C (night/day cycle) and, to prevent lodging, supported by raised grid of nylon line (Pretorius *et al*., 2007). For the duration of the trial, to ensure healthy growth, plants were fertilized twice a week with water-soluble Multifeed-Classic (Efekto®, NPK Analysis 19:8:16 (43), concentration 2.5g/L water), followed by a water suspension of Wonder® 3:2:1, a slow nitrogen release granular fertilizer once in a week, whereas seedling plants were fertilized only with Multifeed-Classic twice a week.

Stem rust pathotypes

Wheat plants were inoculated with *Pgt* pathotypes UVPgt50 (PSKSC, North American classification system, avirulent on *Sr8b, 9g, 21, 24, 26*, *27, 31, 36, 38*,

Tmp, *Kiewiet, Satu and* virulent on *Sr5, 6, 7b, 8a, 9a*, *9b, 9d, 9e*, *9h*, *10, 11, 17, 30,* and *McN),* UVPgt57 (BPGSC, avirulent on *Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 26, 30, 31, 36, 38,* and *Tmp* and virulent on *Sr8a, 9a, 9b, 9d, 9g, 9h, 10, 11, 27, Kiewiet, Satu,* and *McN*), UVPgt59 (TTKSP, avirulent on *Sr9h, 26, 27, 31, 36, Kiewiet, Satu,* and *Tmp* and virulent on *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 21, 24, 30, 38,* and *McN*) and UVPgt60 (PTKST, avirulent on *Sr9h, 21, 26, 27, 36, Kiewiet, Satu,* and *Tmp* and virulent on *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 24, 30, 31, 38,* and *McN*). Race TTKSK avirulent on *Sr9h,* 24, *26, 27, 36, Kiewiet, Satu,* and *Tmp* and virulent on *Sr5*, *6*, *7b*, *8a*, *8b*, *9a*, *9b*, *9d*, *9e*, *9g*, *10*, *11*, *17, 21*, *30*, *31*, *38*, and *McN* was used to screen the 30th ESWYT planted in Kenya (Dr R. Singh, personal communication).

UVPgt50 characterized by virulence for *Sr9e*, became prevalent in SA in the 1980s (Le Roux and Rijkenberg 1987, 1989). UVPgt57 is a South African pathotype with virulence to *SrSatu* occurring in triticale (Olivera *et al*., 2013), whereas UVPgt59 (first detected in the Western Cape at the Tygerhoek experimental farm in 2007) is a member of the Ug99 race group, but with *Sr24* and without *Sr31* virulence (Visser *et al*., 2011). Similar to UVPgt59, UVPgt60 is a member of the Ug99 race group, but with *Sr24* and *Sr31* virulence and was first collected in 2009 from the winter wheat cultivar SST 356 in a rust nursery planted near Greytown, KwaZulu-Natal (Pretorius *et al*., 2010).

Prior to inoculation isolates were retrieved from a -80°C freezer and heat-shocked at 46°C for 6 min. Spores from multiplication pots were dislodged onto paper by gently tapping the leaves. Freshly collected spores were immediately used in experiments whereas any remaining spores were dried over silica gel for 3 days and returned to cryovials at -80°C.

Rust pathotypes were multiplied according to Knott (1989) on susceptible hosts (UVPgt50 on Vernstein, UVPgt57 on Satu, UVPgt59 on LCSr24Ag, UVPgt60 on Federation4*/Kavkaz) to ensure purity. Seeds were planted in 10 cm plastic pots filled with Mikskaar® potting substrate MPS2 and seedlings were fertilized one day before inoculation with 50ml per pot water-soluble Multifeed-Classic (Efekto®, NPK

Analysis 19:8:16 (43), concentration 2.5g/L water). Fertilization was repeated after inoculation when necessary until termination of trials.

Phenotyping

Seedlings – greenhouse trials

Six seeds of each wheat line with five accessions per pot were planted in a 10 cm plastic pot. Substrate and growing conditions were the same as described for rust multiplication. Before inoculation with either of pathotypes UVPgt50, 57, 59 or UVPgt60, seedlings were incubated in a rust-free cubicle at 18-25°C (night/day cycle). Approximately eight days after planting, at the one and a half leaf stage, seedlings were spray inoculated with the designated pathotype. Freshly harvested urediniospores were suspended in mineral oil (Soltrol® 130 Chevron Phillips, Borger, Texas) for spray inoculation (concentration ~3 mg/ml). After inoculation wheat seedlings were dried in a growth cabinet at 25°C for about 1 h before being incubated in the dark in a dew simulation chamber for 16 hours. From the dew chamber seedlings were returned to a growth cabinet fitted with fluorescent growth tubes (Eurolux G135, G13 9W T8 LED) for at least 2 hours. Seedlings were then returned to the greenhouse where previously described growing conditions were maintained. Seedlings were scored 14 days after inoculation according to the 0-4 seedling infection type (SIT) scale (Roelfs *et al*., 1992). Seedling experiments were repeated three times.

Adult plants – greenhouse trials

For all greenhouse trials adult plants (two replications with three to five plants per cone), were inoculated at the onset of flowering (Zadoks growth stage 61) (Zadoks *et al.*, 1974). Wheat plants were grown in sterilized soil in tapered plastic cones (4 x 4 x 10 cm) at 18-25°C in the greenhouse where cones were positioned in a 14 x 7 unit tray, supported by a corresponding, raised grid of nylon line to prevent plants from lodging (Pretorius *et al*., 2007). After seedling emergence plants were fertilized twice with Multifeed® water-soluble fertilizer (19:8:16) NPK plus micronutrients), followed by a water suspension of Wonder® 3:2:1, a slow nitrogen release granular fertilizer, at 7-day intervals for the duration of the trial.

Freshly collected urediniospores were suspended in distilled water (1.25 mg/ml) and Tween 20® (0.03% v/v) and applied to stems according to the method developed by Bender *et al*. (2016, Chapter 3) using a compressed air sprayer similar to the system described by Pretorius *et al*. (2007) for inoculating adult wheat plants with stripe rust. Plants were incubated for 24 h from 13:00 in a plastic dew chamber/tent in a greenhouse cubicle set at 18-25°C (night/day cycle). Upon removal the plants were dried in the same cubicle and growth conditions were maintained as previously described. Stem rust incidence (percentage infected plants), severity and infection reaction type were recorded 14 days after inoculation as host response and for the 30th ESWYT lines, again at 21 days. Severity ratings (Peterson *et al*., 1948) accounted for the whole stem area whereas the infection reaction type (Stubbs *et al*., 1986) (R=resistant, MR=moderately resistant, MS=moderately susceptible and S=susceptible including interpolations) was noted on the last internode. Host reaction type is non-parametric, therefore a constant where R = 0.2, MR = 0.4, MRMS = 0.6, MS = 0.8 and S = 1 (Roelfs *et al*., 1992) was used for analysis thereof and to calculate the coefficient of infection (CI), where severity is multiplied with the constant.

The South African wheat cultivars were inoculated with pathotypes UVPgt57 and UVPgt59. The 24 selected entries from the 30th ESWYT were inoculated with *Pgt* pathotypes UVPgt50, 57 and UVPgt59 in the greenhouse. Flag leaf infection type (IT) was included with infection reaction type and severity on stems when scoring the 30th ESWYT lines as adult plants in the greenhouse to calculate CI. Reaction type on the peduncles, possible *Sr2* occurrence expressed as chlorotic flecking on leaves, leaf tip necrosis (*Ltn*), and stem melanism were recorded when visible. All greenhouse trials were independently repeated for each pathotype.

The Kariega x Avocet S DH mapping population (254 entries), parents and susceptible control were inoculated with UVPgt57 and UVPgt59 in the greenhouse. The trial was independently repeated in the greenhouse with UVPgt59. Flag leaf IT was included with stem rust incidence, severity, infection reaction type and CI when scoring the DH population as adult plants in the greenhouse. The rating of flag leaf infection followed the standard 0-4 scale described by McIntosh *et al.* (1995). For

mapping purposes, it was necessary to convert the scores to a 0-9 scale (Table 4.1).

Adult plants – field trials

Each entry of the cultivar trial, 30th ESWYT, DH mapping population, checks and parental lines were sown as a 1m row with an inter-row spacing of 90cm in a field trial at PANNAR Research Station, Greytown, KwaZulu-Natal, SA early June. Fertilizer (100 kg diammonium phosphate, 50 kg urea and 25 kg KCl per ha) was applied prior to planting. For the DH mapping population the experimental block was replicated, however due to space constraints the cultivar and 30th ESWYT trials were unreplicated in the same season. Stem rust spreader rows (mostly a mixture of Line 37-07, Morocco and McNair) were grown orthogonally between blocks and as trial borders of at least two rows. Furthermore, a rust susceptible entry (either Line 37-07, Morocco or McNair) was included at 10-row intervals in all trials. The trials were irrigated to supplement rainfall and ensure favourable conditions for plant growth and rust development. Spreader rows were spray inoculated with a concentrated inoculum suspension of urediniospores and Soltrol® 130 oil. Following inoculation plants in selected row sections were allowed to dry off for approximately 1 h before covering them with plastic sheeting for 12 hours. Before securing the plastic cover the soil around inoculated plants was watered to promote high humidity inside the plastic sheeting. This process was repeated two to five times per season to establish a homogeneous infection throughout the nursery.

The South African cultivars and the first field trial of the 30th ESWYT were inoculated with pathotype UVPgt59, whereas the second field trial was inoculated with pathotype UVPgt60. Field data for the 30th ESWYT entries, recorded by Dr Ravi Singh in an un-replicated rust nursery inoculated with TTKSK in Kenya, were used to compare with greenhouse data (UVPgt59) and field data from Greytown (UVPgt59 and UVPgt60). For improved comparison the field trial entries inoculated with UVPgt60 were scored twice (early and late). The DH mapping population and control plots of Kariega and Avocet S were evaluated at Greytown for comparison with data from the greenhouse study, where adult plants were inoculated with pathotype UVPgt60. Stem rust responses were recorded for each entry as severity (percentage stem area infected) (Peterson *et al*., 1948) and reaction type (R, MR,

MS and S including interpolations) (Stubbs *et al*., 1986).

Spore carrier

To validate the use of Novec™ 7100 (3M™), an engineered fluid used as an urediniospore carrier by Sørensen *et al*. (2016), it was compared with water in terms of efficiency in greenhouse assessments. Two susceptible lines, one wheat (Line 37-07) and one barley (Gus) were spray inoculated with freshly harvested spores of pathotype UVPgt60. Two concentrations, 1 mg/ml and 0.5 mg/ml, were applied. Previously described water inoculation and incubation methods were followed. Stem rust incidence as a percentage stems infected, severity (Peterson *et al*., 1948), infection reaction type (Stubbs *et al*., 1986) and the number of pustules per 2 cm stem section were recorded 14 days after inoculation.

Data analysis

Data for severity, reaction type (constant value) and CI were analyzed for variance according to a completely randomized design with NCSS Statistical Software System (Hintze, 2007). The relationship between greenhouse and field-derived stem rust scores was determined using Spearman's rank order correlation.

Using the published Kariega x Avocet S maps (Prins *et al*., 2005, 2011) and stem rust traits, composite interval mapping (CIM) was performed with Windows QTL Cartographer V2.5 to: firstly verify whether *Sr5* (6D) and *Sr26* (6A) could be detected, and secondly to determine whether *Lr34/Yr18/Sr57* (7D) contributes towards the stem rust resistance phenotype observed in the population. Mapping was performed by CenGen (Pty) Ltd., Worcester, South Africa.

RESULTS

Seedlings – greenhouse trials

SITs recorded for the four *Pgt* pathotypes on the 24 selected 30th ESWYT entries are shown in Table 4.2. PBW343 was susceptible to pathotype UVPgt60 (*Sr31* virulence), but high levels of resistance were expressed to UVPgt50 (SIT ;;1), UVPgt57(SIT 0) and UVPg59 (SIT 1) (Fig 4.1). All 24 lines produced low SIT (0; to 1 with only 1 plant 1+ in a mixed line) with pathotype UVPgt57 (Table 4.2).

Considering pathotype UVPgt60, 23 of the 24 entries produced susceptible SITs. Although mixed SITs (both R and S) were observed only 30th ESWYT entry 142 expressed resistance with a SIT of 2 for pathotype UVPgt60.

Adult plants

The first symptoms were visible 6 days after inoculation on adult plants in the greenhouse. Response of South African cultivars inoculated with pathotypes UVPgt57 (Fig 4.2 A) and UVPgt59 (Fig 4.2 B) varied from resistant (0R) to susceptible (80S) with mean stem rust incidences of 93% and 98% recorded for the first greenhouse experiment and its independent replicate (Table 4.3). For pathotype UVPgt59 an R^2 = 0.30 (P≤0.05) was obtained for the relationship between greenhouse and field CI scores (Fig 4.3) which was higher for severity (R^2 = 0.81, P≤0.05) than reaction type (R^2 = 0.24) (Fig 4.4). The 0R response of several entries to UVPgt57 was typical of *Sr5* resistance.

Adult plant response of the selected 30th ESWYT entries in Kenya (TTKSK) and greenhouse inoculated plants with pathotype UVPgt59 (TTKSP) are presented in Table 4.4.1. Stems and flag leaves of all plants were uniformly infected with *Pgt*. Reaction types ranged from 20R for PBW343 to 60S for KIRITATI//PBW65/2*SERI.1B (entry 107). However, for this line a more resistant adult plant reaction (20M) was observed in Kenya. Tables 4.4.2 to 4.4.4 demonstrate the flag leaf IT, the reaction type on peduncles, possible *Sr2* and *Ltn* expression and stem melanism recorded 14 days after inoculation. Stem responses (severity and reaction type) were documented at both 14 and 21 days after inoculation. In the greenhouse *Sr2* was expressed only as chlorotic flecking on flag leaves and stems in certain 30th ESWYT lines such as 30th ESWYT-144 and 150 (Fig 4.5) whereas melanism was clearly visible below the node on stems of adult plants in the field (Fig 4.6). Twenty-one days after inoculation response types were confirmed with no changes in severity and minor variations in reaction type, except for the universal susceptible control Morocco where most plants died due to rust infection.

High levels of resistance were expressed to UVPgt57 except for 30th ESWYT-113 (40MSS) in the second replicate. PBW343 was resistant to pathotypes UVPgt50, 57 and UVPgt59 (Fig 4.7) in the greenhouse, but susceptible to UVPgt60 (*Sr31* virulence) (80MSS) in the Greytown field trial. No significant differences (P≤0.05) were observed between the two greenhouse replicates and data were therefore pooled for comparison with field trials and for compiling the graph presented in Fig 4.8. CI calculated on adult plants in the greenhouse were significantly different (P≤0.05) when compared to field trials (Fig 4.8). Although reaction type was statistically (P≤0.05) similar over location and time severity varied (P≤0.05) between greenhouse and field and also for rating time (early or late) (Fig 4.9). *Sr31* virulence is evident from the response types of 30th ESWYT entries 134 to 140 where reaction types varied from very resistant (0 to 5MR) for pathotype UVgt59 to susceptible for both pathotype UVPgt60 (40MS to 80S) and race TTKSK (20MRMS to 40S) in field trials (Fig 4.10).

The flag leaves and stems of greenhouse-grown DH Kariega x Avocet S plants were uniformly infected with *Pgt*. Flag leaves of both the parental lines, Kariega (IT ;1) and Avocet S (IT 0;) were resistant to pathotype UVPgt57, whereas a predominantly susceptible reaction was expressed on the stems of Kariega (70MSS) (Fig 4. 11 A and B). Flag leaves of Kariega (IT Z2++) and Avocet S (IT 1) inoculated with pathotype UVPgt59 expressed intermediate levels of resistance (Fig 4.11 C). Similar results were obtained for UVPgt59 on the stems of Kariega (60MSS), but not for Avocet S, carrying *Sr26*, where a high severity of small pustules were observed (50MR) (Fig 4.11 D). The reaction/infection types of the DH lines ranged from highly resistant to highly susceptible on stems and flag leaves (Fig. 4.11 E and F). Lower flag leaf infection types were noticed on entries with leaf tip necrosis. Segregation ratios between resistant (flag leaf infection types 0; to Z3) and susceptible (flag leaf infection types 3 to 4) DH lines, did not deviate significantly from a three gene model (*Sr5*, *Sr26*, *Sr57*) for UVPgt57 (Chi square$_{7:1}$ = 1.81) (P=0.179) and two gene model (*Sr26*, *Sr57*) for UVPgt59 (Chi Square$_{3:1}$ = 0.54) (P=0.463). Using CIM, flag leaf infection types of UVPgt57 were significantly influenced by regions on chromosomes 6A, 6D and 7D, whereas genes controlling infection types of UVPgt59 mapped to chromosomes 6A and 7D (Table 4.5). As

expected, a low percentage of variance was explained by *Sr57* (7D) for leaf infection types i.e. 1.4% and 6.1% for UVPgt57 and UVPgt59, respectively. Stem severity and reaction type mapped to chromosomes 6A and 6D for UVPgt57 as opposed to only 6A for UVPgt59. The results corresponded with the resistance genes postulated to occur in the population. UVPgt57 is avirulent for *Sr5* and *Sr26* on the Avocet S-derived chromosomes 6D and 6A, respectively, while UVPgt59 is avirulent only for *Sr26*. Neither severity nor reaction type on the stems of the DH lines detected *Sr57* in the greenhouse whereas the 7D region was shown to have a minor effect in reducing severity (R^2 = 6.5%), and the related CI (R^2 = 2.4%), in the field (Table 4.5). The 7D QTL interval also varied for the two leaf infection type scores and the field stem severity score that were significantly detected, with either cssrf6 (*Sr57*-diagnostic) or csLV34 (linked marker) being the most significant associated marker (Fig 4.12). Additional unknown minor QTL were not consistently detected with UVPgt57 and UVPgt59 across the different scores in the greenhouse, and similar to *Sr57*, the phenotypic variance explained by them were ≤ 1.7%. Minor QTL of unknown identity were more consistently detected in the field score with UVPgt60. The chromosome regions of the minor resistances were not the same across the three pathotypes tested and were not considered further.

Infection frequency (% stems infected/incidence) for inoculation with water as a carrier was slightly lower than for Novec 7100 and this was more pronounced with the barley cultivar Gus, where 100 % of inoculated stems were infected (Table 4.6). Although not all stems of Line 37-07 were infected with the higher Novec 7100 concentration, more pustules developed on the stems (Fig 4.13 A). Smaller pustule sizes were formed on stems of Gus (MS), whereas Line 37-07 (S) demonstrated large, fully compatible pustules (Fig 4.13 B and C).

DISCUSSION

The main objective of the present study was to verify the recently developed greenhouse inoculation method (Chapter 3; Bender *et al*., 2016) using South African wheat cultivars, selected entries from the 30th ESWYT and a DH mapping population by comparing adult plant rust data recorded in the greenhouse with field data. APR is an important component of breeding wheat for rust resistance

(Lagudah, 2011, Singh *et al*., 2011, 2015, Agenbag *et al*., 2012, Njau *et al*., 2013, Tsilo *et al*., 2014, Yu *et al*., 2014) due to the general perception that it may be more durable. Therefore, accuracy and repeatability of APR phenotyping can contribute to the timely development of resistant varieties. Although, assessment of rust infection in the field will always be essential to unravel genotype by environment interactions, this newly developed greenhouse screening method presents a controlled environment to study infection responses in APR. Control over factors such as rust pathotype, quality and quantity of inoculum, host growth stage at time of inoculation, a single infection cycle and fixed incubation period, as well as temperature and light, help to minimize variation when comparing phenotypic responses. Host responses to *Pgt* are often influenced by temperature. High temperatures inhibit the expression of resistance by *Sr6*, *Sr10*, *Sr15* and *Sr17* (Chen *et al*., 2018, Gao *et al*., 2019), whereas *Sr13* and *Sr21* are more effective at higher temperatures (Chen *et al*., 2018, Zhang *et al*., 2017).

Pretorius *et al*. (2000) pioneered the concept of improving and accelerating adult plant rust phenotyping in a controlled environment when they showed that APR to leaf rust and stripe rust was clearly expressed in flag leaves of plants grown under continuous light and inoculated 28 days after planting. However, in 2007, Pretorius *et al*. achieved better expression of stripe rust APR by raising more vigorous plants in the greenhouse. Development of wheat stripe rust symptoms is influenced by the age of leaves, with recently exposed leaves found more prone to infection and comparatively more susceptible than their older counterparts (Ramburan *et al*., 2004, Hickey *et al*., 2012). The greenhouse-grown mini adult plants used by Pretorius *et al*. (2007) were acceptable for wheat stripe rust screening. However, improved plant development including thicker and rigid stems, is needed for stem rust assessment.

Singh (1992) reported that low infection types of wheat leaf rust were better expressed on older leaves, which supports the approach of phenotyping adult plants at a predetermined growth stage. Furthermore, flowering was identified as the best growth stage for reliable greenhouse observations for *Sr2* expression (Sunderwirth and Roelfs, 1980). Lower CI’s were observed on cultivars with the

APR gene *Sr2* such as Steenbras (Pretorius *et al*., 2012), Krokodil and Tankwa confirming that infection of adult plants in a greenhouse will be an additional tool for stem rust resistance assessments. Less research has been done on the expression of stem rust APR under greenhouse conditions. In a study by Eaton *et al*. (1984) the authors concluded that stem rust APR expressed in the field cannot be predicted under greenhouse conditions. However, the data presented in this study indicate that greenhouse responses can accurately predict APR in the field. Through applying one infection cycle and the early visibility of uredinia on stems (6 days after inoculation) this method further presents the opportunity to calculate latent period and pustule size for macro- and microscopic components of resistance.

The differences observed between field and greenhouse CI are an indication of the influence of genotype by environment interactions. The most commonly used method to assess stem rust disease severity is visual ratings, always providing the risk of repeatability due to subjectivity of the person involved. Furthermore, disease pressure in field trials may vary in and between seasons influencing the severity of ratings. Variables may include the date of disease onset, host growth stage, disease pressure and development, as well as the time duration for the disease to peak (Hamilton and Stakman, 1967, Boshoff *et al*., 2019). Repeated infection cycles in the field through polycyclic spore production versus the single inoculation and infection cycle in the greenhouse might explain most variation observed in severity, while host reaction type ratings were clearly similar between greenhouse and field trials. Bancal *et al*. (2007) reported that lines displaying very high stripe rust severity tended to show lower stem rust severities because of the reduced photosynthetic area for *Pgt* infection on stems. Previous research by Case *et al*. (2017) included only severity for analysis. However, the moderate infection levels recorded in their trials might have contributed to a good correlation among nurseries, compared to an immense inoculum pressure for both stem and stripe rust usually experienced in field trials at the Greytown trial site. The deliberate selection of mostly susceptible lines for technique development in the greenhouse unfortunately influenced the correlation outcome. Overall, the APR results of the greenhouse developed method is comparable with those recorded in the field trials and it is considered worthwhile

to complement field studies with greenhouse studies. Greenhouse evaluation can be applied for a reasonable number of entries, is not growth season dependent and can include more than one cycle of evaluation per year. Provided infrastructure exists, data can be generated faster and less costly and contribute to a better understanding of the expression of a particular rust resistance source.

SIT results for the 30th ESWYT entries are in agreement with APR data generated in the greenhouse and field, expressing mostly susceptible responses with UVPgt60. Only one line showed resistance at the seedling stage to UVPgt60 (ESWYT 142, SIT 2), but was considered susceptible in the field to UVPgt60 despite the fact that it also tested positive for the *Sr2* resistance gene. The reason for this discrepancy needs further clarification, although a similar trend in seedling resistance as opposed to field susceptibility was reported for *SrTmp* (Boshoff *et al*., 2019). The expression of all-stage resistance genes such as *Sr5* and *Sr26* was clearly visible during the adult plant screening, however seedling assays are more efficient if major gene confirmation is the only objective. Should the interaction between resistance genes in adult plants be the priority of a study, the currently described method will be more supportive. It is unlikely that greenhouse phenotyping will replace field testing as resistance conferred by *Sr57*, was not reliably detected. Interestingly, when *Sr57* was detected in the adult plant leaf infection type scores versus the field scores, using different pathotypes, the significant QTL region varied slightly, with the same interval not being consistently detected. Therefore, this upgraded greenhouse technique was validated with both the SA cultivars and 30th ESWYT and an extended Kariega x Avocet S population where all three major stripe rust resistance QTL were detected (Prins *et al*., 2011). Additional analysis of the technique with Novec 7100 fluid as a spore carrier supports the use thereof for rapid screening and therefore accelerating phenotyping of stem rust resistance in breeding.

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Table 4.1: Conversion of flag leaf stem rust infection types to a 0-9 scale.

Infection type	Scale
0; to ;	1
;c to ;cn	2
Z;1	3
;1c to ;1+	4
Z2 to 2=*	5
2 to 2+3	6
Z3 to 3=	7
3 to 3++	8
4	9

* including c and n

Table 4.2: Seedling infection types 14 days after inoculation according to Roelfs *et al.* (1992) produced by *Puccinia graminis* f. sp. *tritici* pathotypes UVPgt50, 57, 59 and UVPgt60 on 24 30th ESWYT entries.

Entry	UVPgt50	UVPgt57	UVPgt59	UVPgt60
Line37-07*	3+	3++	3+	4
PBW343	;;1	0;	1	3++
104	1p;1,2++3	;;1	0;,3	;,4
106	;,3	0;	0;	;,3
107	;1,2	;1	;1,1p2	3++4
109	0;	0;	;;1	3++
110	0;	0;	0;,1p3	4
111	0;	;;1	;;1	4
112	0;,2+	0;	;1	;1,1p4
113	0;,2++	;;1	;;1	4
114	0;,;1	;	;1	3++
115	0;,1p3	0;,1p1+	0;,1+,3	4
116	0;	0;	;;1	3c
117	0;	;;1	0;	3
118	0;	0;	;,1	;,4
127	;;1	0;	0;,2	;,4
128	X	;;1	0;	;,3
129	;1,2+	;;1	0;	3++
130	0;	0;	0;	3
141	0;	0;	0;	3++
142	0;	;;1	;;1	2
143	0;,;1	0;	;;1	4
144	0;	0;	0;	3
149	0;	0;	;	3++
150	3	1	3	3++
Morocco*	4	4	4	4

Mixed infection type scores: e.g. 1p;1,2++3 where 1 plant had a score of ;1 and the rest a score of 2++3.

*Controls: Line 37-07 and Morocco (universal susceptible)

Table 4.3: Stem rust response as severity and reaction type, produced by *Puccinia graminis* f. sp. *tritici* pathotypes UVPgt57 and UVPgt59 on adult plants of 20 South African cultivars under greenhouse and field evaluation (Greytown).

Cultivars	Greenhouse				Field
	UVPgt57		UVPgt59		UVPgt59
	1st Replicate	2nd Replicate	1st Replicate	2nd Replicate	
PAN 3434	20MRMS	20MRMS	50S	70MSS	20MSS
SST 57	0R	0R	40MS	40MR	30MSS
Baviaans	30MS	20MSS	70MSS	50MS	20S
SST 825	40MRMS	30MRMS	70S	80S	60MSS
SST 88	tR	5R	70S	80S	50S
SST 835	20R	20MRMS	60MS	60MS	30S
SST 822	0R	0R	80MS	50MS	30MSS
SST 027	0R	0R	5MS	30MSS	40MSS
CRN 826	0R, 5S	0R, tS	10S	80S	70MSS
SST 015	30MR	50MR	40MRMS	50MRMS	5R
Steenbras	0R	0R	0R	0R	0R
Kariega	50MSS	40MSS	50S	60S	70S
SST 047	0R	0R	30R	0R	5R
PAN 3492	10R	5R	20MSS	60S	30MS
Marico	10R	5R	50S	50S	80S
PAN 3408	30MS	30MSS	70S	70S	70MSS
SST 876	tMR	5MR	50MSS	80MSS	90S
Olifants	0R, 40MR	0R	60S	50MS	60MSS
Krokodil	10RMR	20RMR	40MRMS	60MR	60MS
Tankwa	0R	0R	10R	10R	0R
Morocco	70S	80S	80S	70S	80S

Severity (Peterson *et al*., 1948) and reaction types (Stubbs *et al*., 1986) were determined 14 days after inoculation in the greenhouse

Table 4.4.1: Adult plant host responses of the 30th ESWYT entries to *Puccinia graminis* f. sp. *tritici* pathotypes UVPgt59 (TTKSP), in the greenhouse and first field trial in Greytown, UVPgt60 (PTKST) in the second field trial at Greytown and race TTKSK in Kenya.

	Greenhouse UVPgt59	Greytown UVPgt59	Greytown UVPgt60		Kenya TTKSK
Entry		**Early 25 Oct**	**Early 25 Oct**	**Late 4 Nov**	**11 Oct**
Line 37-07*	50S	30MSS	30S	80S	no data
PBW343	30MR	15MS	30MSS	80MSS	no data
103		5MR	10MSS	40MRMS	30MS
104	50MSS	20MS	30MSS	50MSS	40MSS
105		10MS	20MSS	50MSS	50MSS
106	50MSS	TMRMS	20MS	60MS	30MSS
107	30MSS	5MS	15MRMS	20MR	20MSS
108		5MR	15S	50MRMS	20MSS
109	40MS	15S	80S	100S	40MSS
110	30MSS	5MR	10MSS	40MRMS	10MSS
111	40MRMS	5MR	30MSS	80MSS	30M
112	30MS	5MS	30MSS	70S	30MSS
113	30MS	20MS	30MSS	50MSS	20MSS
114	30S	5MS	20MS	70MSS	20MSS
115	50MSS	5MS	15MRMS	40MRMS	20MSS
116	30MRMS	5MR	10MRMS	30MRMS	10MSS
117	50MSS	30MSS	40S	90S	30MSS
118	30MRMS	TMS	10MR	50MR	10MS
119		TMS	10MR	60MRMS	20MSS
120		10MRMS	20MR	50MRMS	20MSS
121		TRMR	5R	20R	5R
122		0	0R	10R	5R*
123		TR	0R	20RMR	5R
124		0	0R	20RMR	10M
125		TR	TMR	20RMR	5RMR
126		TR	5RMR	20RMR	15M
127	20MR	5MS	TMRMS	30MR	30MSS
128	30MR	10MRMS	TRMR	20RMR	20MSS
129	30MS	10MRMS	15S	50MSS	20MSS
130	40MSS	20MSS	30MS	70S	30MSS

	Greenhouse UVPgt59	Greytown UVPgt59	Greytown UVPgt60		Kenya TTKSK
Entry		**Early 25 Oct**	**Early 25 Oct**	**Late 4 Nov**	**11 Oct**
131		5MR	30MS	60MSS	30S
132		15MRMS	30MS	60MSS	30MSS
133		TMS	20MS	70MSS	40S
134		TMS	15MRMS	50MS	30S
135		0	30MRMS	50MRMS	40S
136		5MR	30S	80S	30S
137		5RMR	30MRMS	50MS	50MSS
138		TR	5MSS	40MS	5MS
139		TR	70S	100S	40M
140		TR	70S	100S	20M
141	20MS	TMS	30MS	60MS	20M
142	50MSS	20MRMS	80MSS	100MS	40MR
143	20MS	10MS	30MS	50MS	30MSS
144	20MR	TMR	10MRMS	50MRMS	10MS
145		TMS	10MSS	30MS	30MSS
146		5MS	10S	40S	40MSS
147		TMR	15MS	40MRMS	30M
148		TMR	40S	90S	30MSS
149	40MRMS	TMR	20MRMS	50MS	20M
150	20MRMS	TMS	30S	70S	20M
Morocco*	50MSS	20S		100S	

*Controls: South African Line 37-07 and Morocco (universal susceptible)
Severity (Peterson *et al*., 1948) and reaction types (Stubbs *et al*., 1986) were determined 14 days after inoculation in the greenhouse

Table 4.4.2: Adult plant host responses to pathotype UVPgt50 of *Puccinia graminis* f. sp. *tritici* on wheat leaves, stems and peduncles of selected lines of the 30th ESWYT 14 and 21 days after inoculation in the greenhouse (left 1st replicate and right 2nd replicate).

Days after inoculation	14						21	14					21
Entry	Leaf [a]IT	Stem response	Peduncle reaction type	[b]*Sr*	[c]Ltn	[d]Sm	Stem response	Leaf [a]IT	Stem response	Peduncle reaction type	[c]Ltn	[d]Sm	Stem response
Line 37-07*	3	30S	S				30S	dead	50MSS	S			dead
PBW343	0	5R	0	*Sr31*			5R	;1-	20R	0			20R
104	3	30S	0				30S	2+	30MSS	0			30MSS
106	Z2+	40MS	0	*Sr2*			40MSS	2-	20MRMS	0	Ltn	sm	20MS
107	;1+	5MS	0	*Sr2*			5MSS	0;	5RMR	0	Ltn		5RMR
109	2	70MRMS	0	*Sr2*			70MRMS	;1	30MR	M	Ltn	sm	30MRMS
110	2+c	30S	0	*Sr2*			30S	2	10MS	0	Ltn		10MS
111	3	20MSS	0	*Sr2*			20MRMS	Z2	30MRMS	M	Ltn		30MRMS
112	3	25MSS	0				25S	2+	25MS	TM			25MS
113	Z2+	30MRMS	0	*Sr2*			30MS	Z;1	15MRMS	TM	Ltn		15MS
114	3	10S	0		Ltn		10S	3	5S	0			5S
115	3	30S	0	*Sr2*			30MSS	;	10MS	0	Ltn		10MSS
116	2	15MRMS	0	*Sr2*	Ltn	sm	15MRMS	;	TMRMS	0	Ltn		TMR
117	Z2	50MS	0	*Sr2*	Ltn		50MRMS	;1	20MRMS	0	Ltn	sm	20MRMS
118	;1	30MR	0		Ltn		30MR	;	5R	0	Ltn		5R
127	;	20R	0	*Sr2*		sm	20R	;	10R	R	Ltn	sm	10R
128	;	15R	0	*Sr2*		sm	15MR	;	15R	M	Ltn	sm	15R
129	0;	20R	0	*Sr2*		sm	20R	Z;1	30MR	M	Ltn	sm	30MR
130	2+	40S	0				40S	2	50S	0			50MSS
141	;1	10MRMS	0	*Sr2*			10MRMS	;1,3	0R,30MS	0,0			10R,30MSS
142	2+	60MSS	0	*Sr2*			60MSS	Z;1	40MR	S	Ltn		40MR
143	2++3	20MRMS	MR	*Sr2*			20MRMS	Z2	20MR	M	Ltn	sm	20MR
144	2-	40MR	0	*Sr2*			40MR	2	10RMR	M		sm	10RMR
149	2	40MR	MS	*Sr2*			40MR	;	10MR	TM	Ltn		10MR
150	0;	30MR	0	*Sr2*	Ltn		30MR	dead	20RMR	0			dead, RMR
Morocco*	3	10S	S				10S	3	60S	S			dead, S

[a] IT = Infection type, [b] *Sr* = stem rust gene, [c] Ltn = leaf tip necrosis, [d] Sm = Stem melanism

* Controls: South African Line 37-07 and Morocco (Universal susceptible)

Severity (Peterson *et al*., 1948) and reaction types (Stubbs *et al*., 1986) were determined 14 and 21 days after inoculation in the greenhouse

Table 4.4.3: Adult plant host responses to pathotype UVPgt57 of *Puccinia graminis* f. sp. *tritici* on wheat leaves, stems and peduncles of selected lines of the 30th ESWYT 14 and 21 days after inoculation in the greenhouse (left 1st replicate and right 2nd replicate).

Days after inoculation	14						21	14				21
Entry	Leaf [a]IT	Stem response	Peduncle response	[b]*Sr*	[c]Ltn	[d]Sm	Stem response	Leaf [a]IT	Stem response	Peduncle response	[d]Sm	Stem response
Line 37-07*	3	10S	S				10S	dead	dead			dead
102	0;	0R	0		Ltn		0R	;	0R	0		0R
104	;	0R	0	*Sr2*	Ltn		0R	0	tR	0		tR
106	;	tR	0	*Sr2*			tR	;	0R	0		0R
107	0;	tR	0	*Sr2*	Ltn		tR	;	0R	0		0R
109	;1	30RMR	0	*Sr2*		sm	30RMR	;	30RMR	M	sm	30RMR
110	;	tR	0	*Sr2*			tR	;	0R	0		0R
111	;	0R,30MRMS	0,S				0R,30MRMS	;	0R	0		tR
112	;;c	0R	0	*Sr2*	Ltn		0R		0R	0		0R
113	;	15MRMS	0	*Sr2*	Ltn		15MS		40MS	S		40MSS
114	2	10S	0		Ltn		10S	0	15MRMS	S		15MS
115	0;	0R	0	*Sr2*			0R	;	5RMR	0		5MSS
116	0;	0,10R	0	*Sr2*	Ltn		0,10R	;,;	tR,10R	0,S		tR,10R
117	0;	0R,15MR	0				0R,15MR	Z;1	20RMR	M	sm	20RMR
118	0	5R	0				5MRMS	;	0R	0		0R
127	;	10R	0	*Sr2*		sm	10R	Z;1	40R	M		40RMR
128	;	30R	0	*Sr2*	Ltn		30MR	;	40R	S		40RMR
129	;;1	30R	0	*Sr2*			30R	;	20R	M		20RMR
130	;	0R	0	*Sr2*			0R	;	0R	0		0R
141	0;	0R	0				0R	;	0R	0		0R
142	;	10MR	0	*Sr2*	Ltn		10MRMS	;	20MR	S		20MR
143	;	0R	0	*Sr2?*			0R	;	0R	0		0R
144	;c	tR	0	*Sr2*	Ltn		tR	;	0R	0		0R
149	;	0R	0		Ltn		0R	;	0R	0		0R
150	;	5R	0	*Sr2*	Ltn		5R	;	20MR	M	sm	20MRMS
Morocco*	3	20S	Very S				20S	3	70MSS	S		dead

[a] IT = Infection type, [b] *Sr* = stem rust gene, [c] Ltn = leaf tip necrosis, [d] Sm = Stem melanism

* Controls: South African Line 37-07 and Morocco (Universal susceptible)

Severity (Peterson *et al*., 1948) and reaction types (Stubbs *et al*., 1986) were determined 14 and 21 days after inoculation in the greenhouse

Table 4.4.4: Adult plant host responses to pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici* on wheat leaves, stems and peduncles of selected lines of the 30th ESWYT 14 and 21 days after inoculation in the greenhouse (left 1st replicate and right 2nd replicate).

Days after inoculation	14					21	14				21
Plot	Leaf [a]IT	Stem response	Peduncle response	[b]*Sr*	[c]Ltn	Stem response	Leaf [al]T	Stem response	Peduncle response	[b]Sm	Stem response
Line 37-07*	4	50S	S			50MS	3	50S	S		50 dead
102	;;1	30MR	R	*Sr31*		30RMR	0	20R	M		20R
104	2-	50MSS	0			50MSS	2	40S	S		40S
106	2	50MSS	0			50MSS	;	30MR	0		30MRMS
107	2+	30MSS	0			30MSS	2	50MS	0	sm	50MS
109	2	40MS	0			40MRMS	2	50MS	S	sm	50MS
110	3	30MSS	0			30MSS	;1	50S	0		50MS
111	2+	40MRMS	0	*Sr2*		40MR	2+	30MRMS	S	sm	30MS
112	;1	30MS	0	*Sr2*	Ltn	30MRMS	2	25MRMS	S		25MS
113	;1+	30MS	0	*Sr2*	Ltn	30MS	;	10MS	S		10S
114	3	30S	R			30S	3	30S	0		30S
115	4	30S	R			30S	3	50MSS	0		50MSS
116	0	50S	R		Ltn	50MRMS	;;1	30MRMS	S	sm	30MRMS
117	1+	50MSS	0	*Sr2*	Ltn	50MSS	Z2	30MRMS	S		30MRMS
118	2+	30MRMS	MS		Ltn	30MS	12	40MRMS	S		40MRMS
127	0	20MR	MR		Ltn	20MR	;	30MRMS	S	sm	30MRMS
128	;;1	30MR	0		Ltn	30MR	;	50MS	MS	sm	50MS
129	Z2	40MRMS	0		Ltn	40MR	;	30MS	S	sm	30MS
130	1+	40MS	0	*Sr2*	Ltn	40MS	2	40MSS	0		40MSS
141	3-	20MS	MS			20S	2+	60S	S		60S
142	2-	30MR	0		Ltn	30MR	2+	50MSS	S		50MSS
143	3	20MS	MS			20MS	Z2+	50MS	S	sm	50MS
144	2-	20R	MR	*Sr2*	Ltn	20R	;,Z;1	5MR,MS	MS	sm	5MR,30MRMS
149	2	40MRMS	S		Ltn	40MRMS	Z;;1	40MRMS	S		40MRMS
150	0	20MRMS	0	*Sr2*	Ltn	20MRMS	dead	30RMR	M		30dead
Morocco*	2	50MSS	S			50MSS	dead	50MSS	S		50dead

[a] IT = Infection type, [b] *Sr* = stem rust gene, [c] Ltn = leaf tip necrosis, [d] Sm = Stem melanism

* Controls: South African Line 37-07 and Morocco (Universal susceptible)

Severity (Peterson *et al*., 1948) and reaction types (Stubbs *et al*., 1986) were determined 14 and 21 days after inoculation in the greenhouse

Table 4.5: Adult plant stem rust resistance QTL detected by composite interval mapping in a double haploid wheat mapping population evaluated in the greenhouse and field.

		Leaves			Stems							
			Infection type		Severity		Reaction type		Coefficient of Infection			
BPGSC[a] Chr[c]	**QTL Interval**	**Associated Marker**	**LOD**	**R ²**	**LOD**	**R ²**	**LOD**	**R ²**	**LOD**	**R ²**	**Gene origin**	**Resistance gene**
6A	wPt6951-wmc163.2	Sr26.43	13.1	7.7	6.2	3.9	24.9	21.7	9.0	7.6	AvS	*Sr26*
6D	cfd75-wPt3879	psp3200	62.4	60.8	32.2	26.1	20.9	17.5	17.7	16.0	AvS	*Sr5*
7D	barc352-cssrf6	cssrf6	2.7	1.4	NS	NS	NS	NS	NS	NS	Kariega	*Lr34/Yr18/Sr57*
TTKSP[a] Chr	**QTL Interval**		**LOD**	**R ²**	**LOD**	**R ²**	**LOD**	**R ²**	**LOD**	**R ²**	**Gene origin**	**Resistance gene**
6A	wPt6951-wmc163.2	Sr26.43	8.6	7.4	14.4	22	29.5	15.2	37.5	46.9	AvS	*Sr26*
7D	cssrf6-gwm111.1	csLV34	8.0	6.1	NS	NS	NS	NS	NS	NS	Kariega	*Lr34/Yr18/Sr57*
PTKST[b] Chr	**QTL Interval**				**LOD**	**R ²**	**LOD**	**R ²**	**LOD**	**R ²**	**Gene origin**	**Resistance gene**
6A	wPt6951-wmc163.2	Sr26.43			19.5	23.2	44.7	52.7	31.7	39.1	AvS	*Sr26*
7D	cssrf6-gwm295	csLV34			6.3	6.5	NS	NS	2.6	2.4	Kariega	*Lr34/Yr18/Sr57*

[a]UVPgt57 and 59 stem rust pathotypes were used for greenhouse inoculation
[b]UVPgt60 stem rust pathotype was used for field inoculation
[c]Chromosome
*QTL significantly present with LOD score >2.2 (P=0.05) after 1000 permutation

Table 4.6: Stem rust incidence, severity and host response type, produced by pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici* on adult plants of wheat Line 37-07 and the barley cultivar Gus in the greenhouse.

Line	Carrier	Spore concentration mg/ml	Infection frequency %	Nu of pustules on 2cm stem area	Host response range
Line 37-07	Water	1	89	6	TS, 5S, 10S
	Water	0.5	79	4	TS, 5S, 10MS, 10S
	Novec	1	95	>40	30-50MSS, 20S
	Novec	0.5	100	24	40-50MSS,10S, 50S
Gus	Water	1	67	2	TMS, 5MSS
	Water	0.5	66	1.4	TMRMS, TMS
	Novec	1	100	9	TMRMS, T-10MSS, 5S
	Novec	0.5	100	19	T-20MS, 50MRMS, T-20S

Severity (Peterson *et al*., 1948) and reaction types (Stubbs *et al*., 1986) were determined 14 days after inoculation in the greenhouse

Figure 4.1: Seedling infection types (SIT) for PBW 343, fourteen days post inoculation, produced by pathotypes UVPgt50, 57, 59 and UVPgt60 (left to right) of *Puccinia graminis* f. sp. *tritici*.

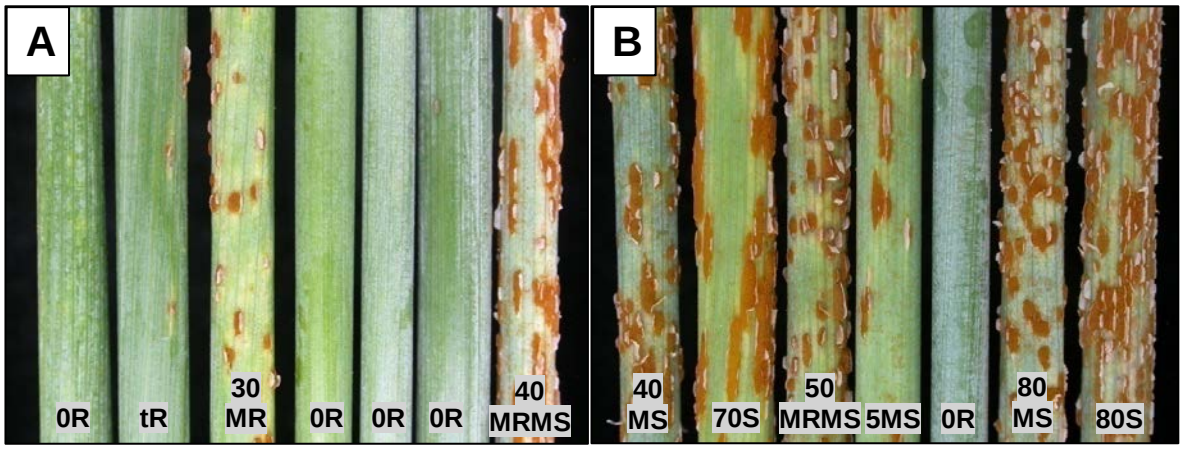

Figure 4.2: Adult plant responses to pathotype UVPgt57 (A) and UVPgt59 (B) of *Puccinia graminis* f. sp. *tritici* on wheat stems in the greenhouse for SST 57, SST 88, SST 015, SST 027, SST 047, SST 822 and SST 825 (left to right) recorded 14 days after inoculation.

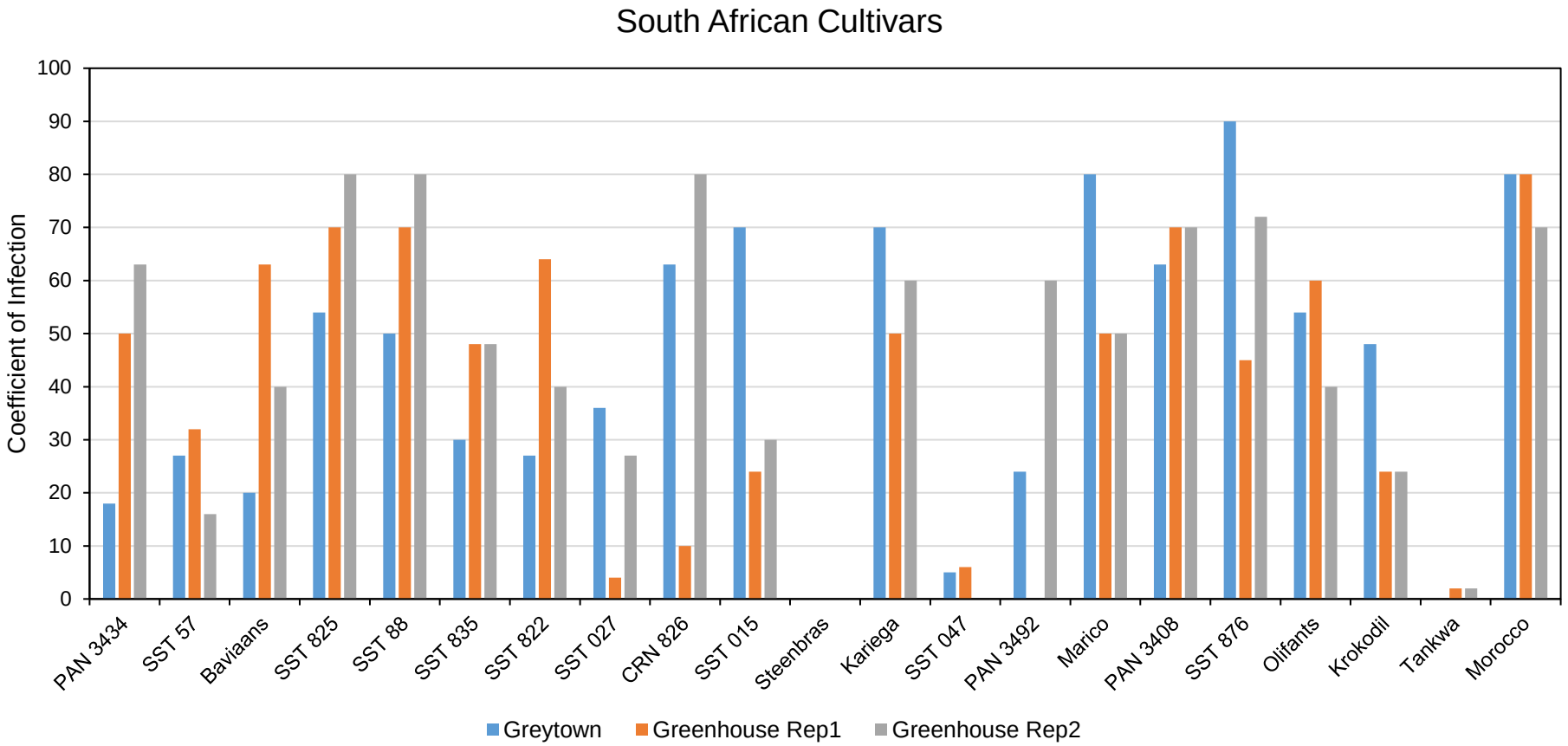

Figure 4.3: Comparison of Coefficient of Infection for 20 South African cultivars planted in a field trial near Greytown, KwaZulu-Natal and two trial replications planted in the greenhouse. The field trial and greenhouse trials were inoculated with urediniospores of pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici*.

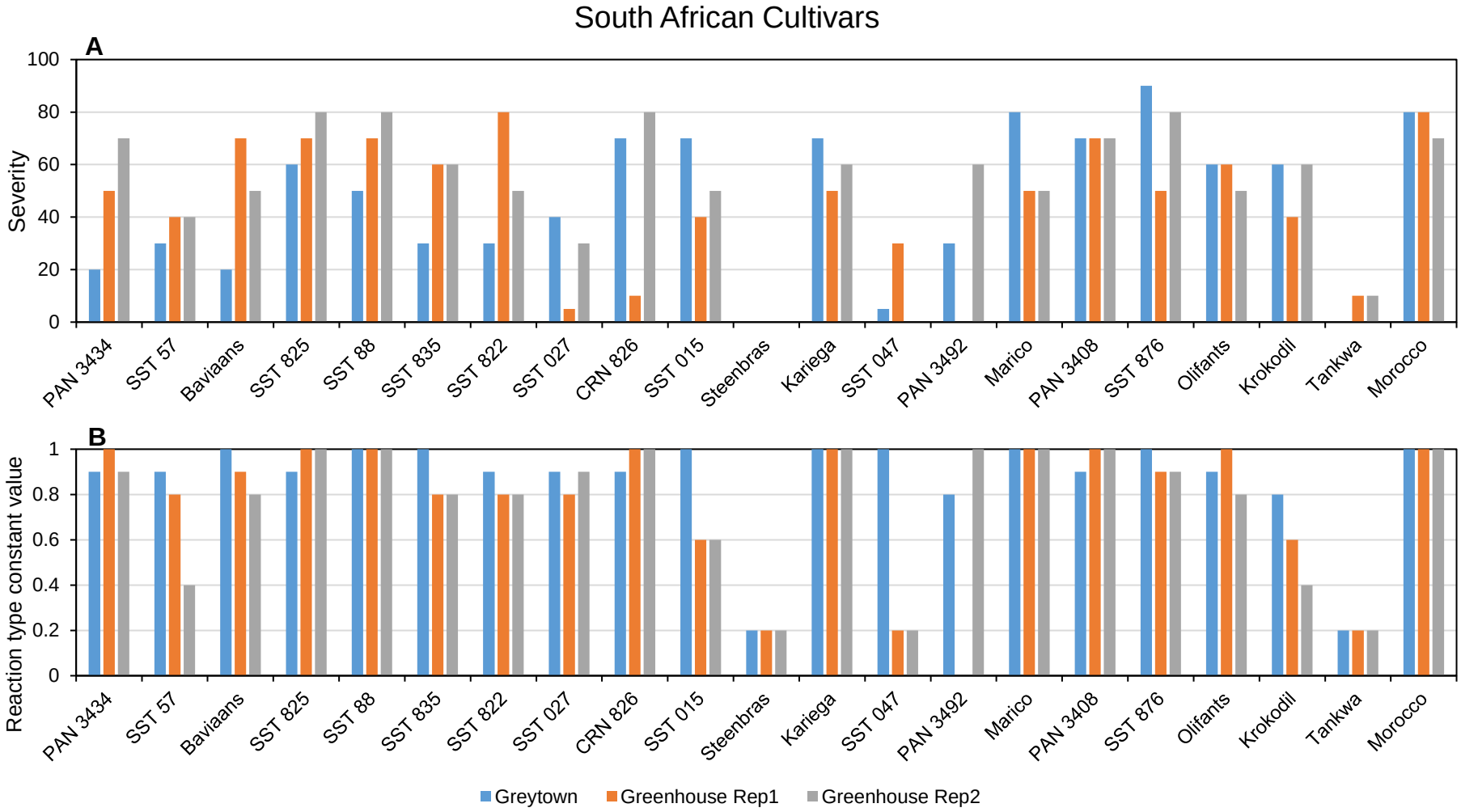

Figure 4.4: Severity (A) and stem rust reaction type (constant value) (B) on adult plants for 20 South African cultivars planted in a field trial near Greytown, KwaZulu-Natal and two trial replications planted in the greenhouse. The field trial and greenhouse trials were inoculated with urediniospores of pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici*.

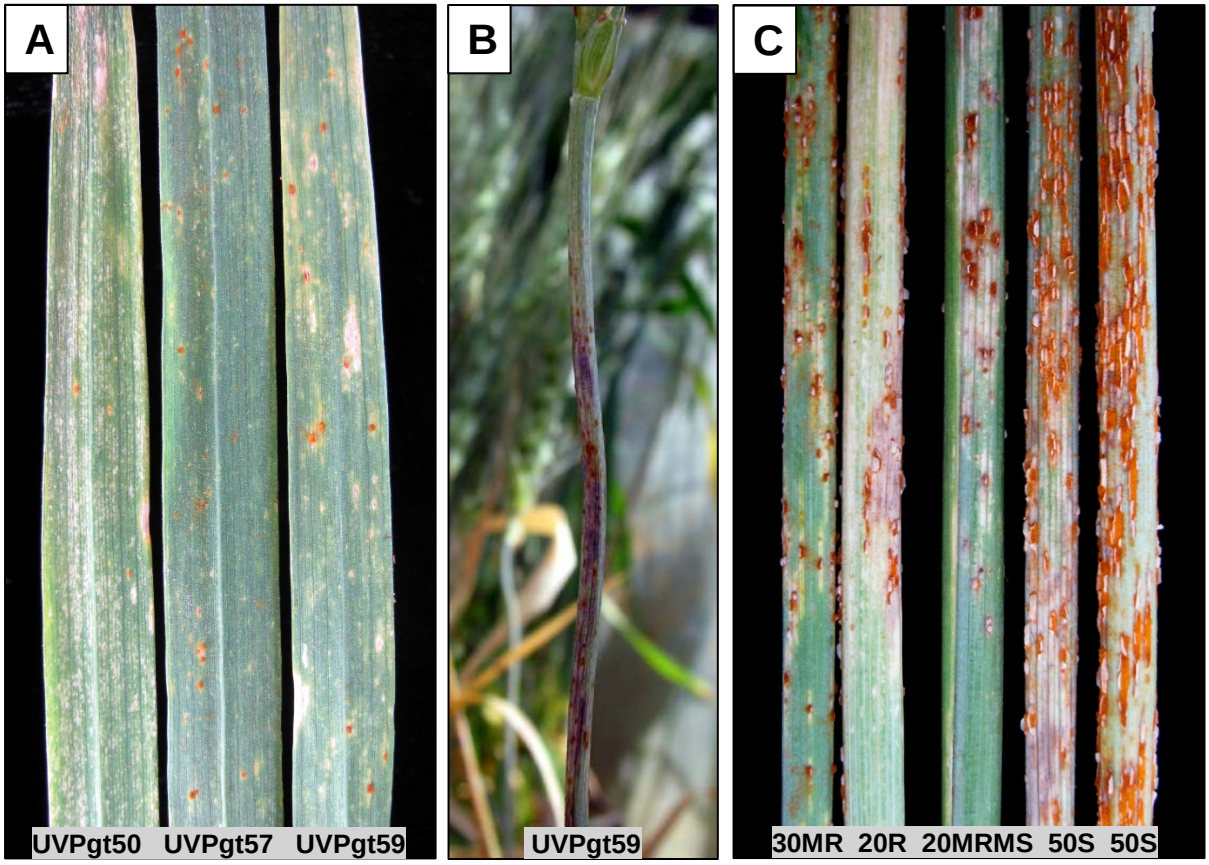

Figure 4.5: Assumed *Sr2* expression on flag leaves (A) and peduncle (B) for entry 150 of the 30th ESWYT, inoculated with either pathotype UVPgt50, 57 or UVPgt59. Stems (C) of 30th ESWYT entries 102 (PBW343), 144 (*Sr2*), 150 (*Sr2*), 116 and control (Line 37-07) (left to right) inoculated with pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici* 14 days after inoculation in the greenhouse trial.

Figure 4.6: Assumed *Sr2* expression on stems of adult wheat plants 30th ESWYT entry 150 (increased susceptibility above nodes) in the greenhouse (A) and field (B), also showing purple discolouration, inoculated with pathotype UVPgt59 of *Puccinia graminis* f. sp. *tritici*.

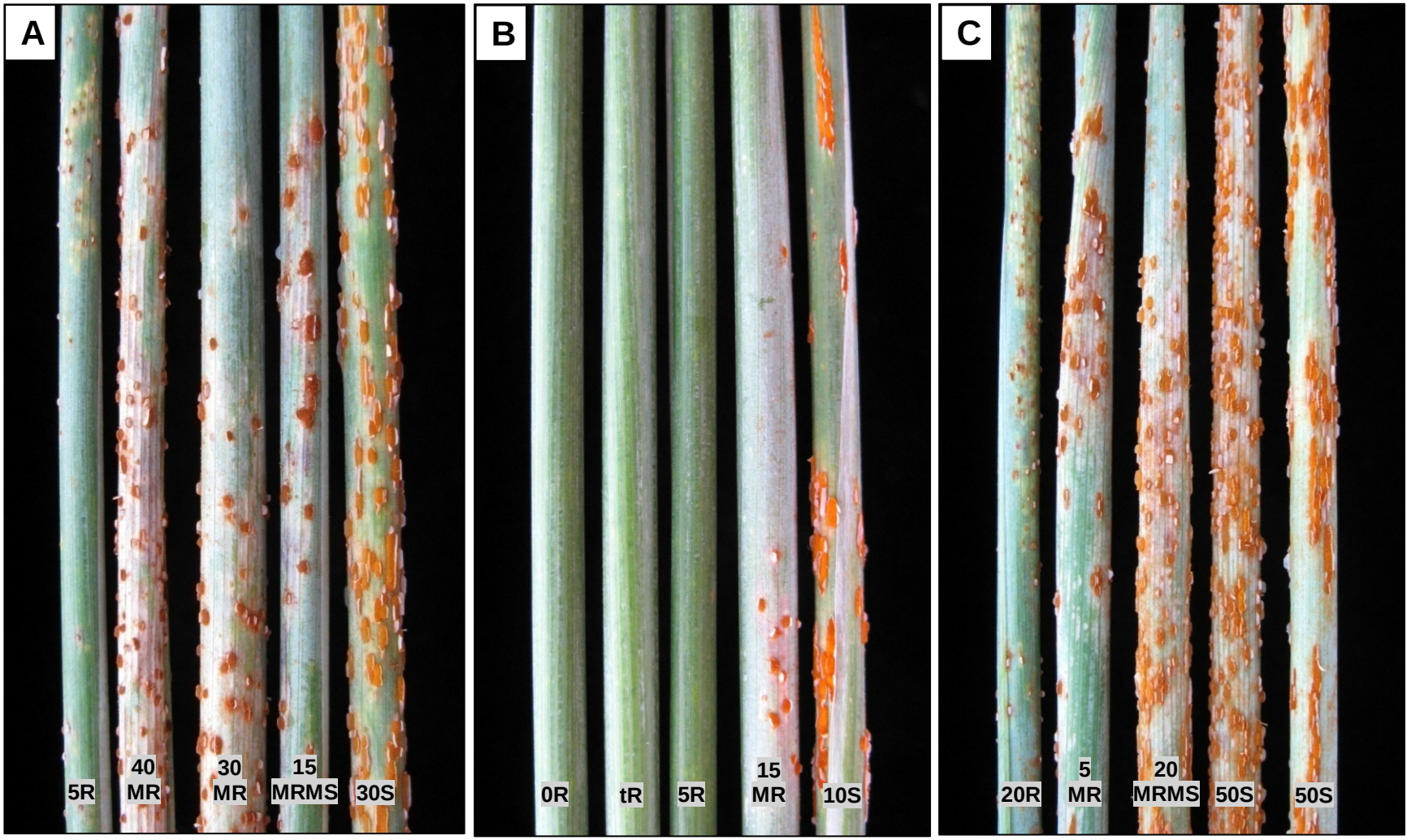

Figure 4.7: Adult plant response to pathotypes UVPgt50 (A), UVPgt57 (B) and UVPgt59 (C) of *Puccinia graminis* f. sp. *tritici* on wheat stems of 30th ESWYT entries 102 (PBW343), 144, 150, 116, and control (Line 37-07) (left to right) recorded 14 days after inoculation in the greenhouse.

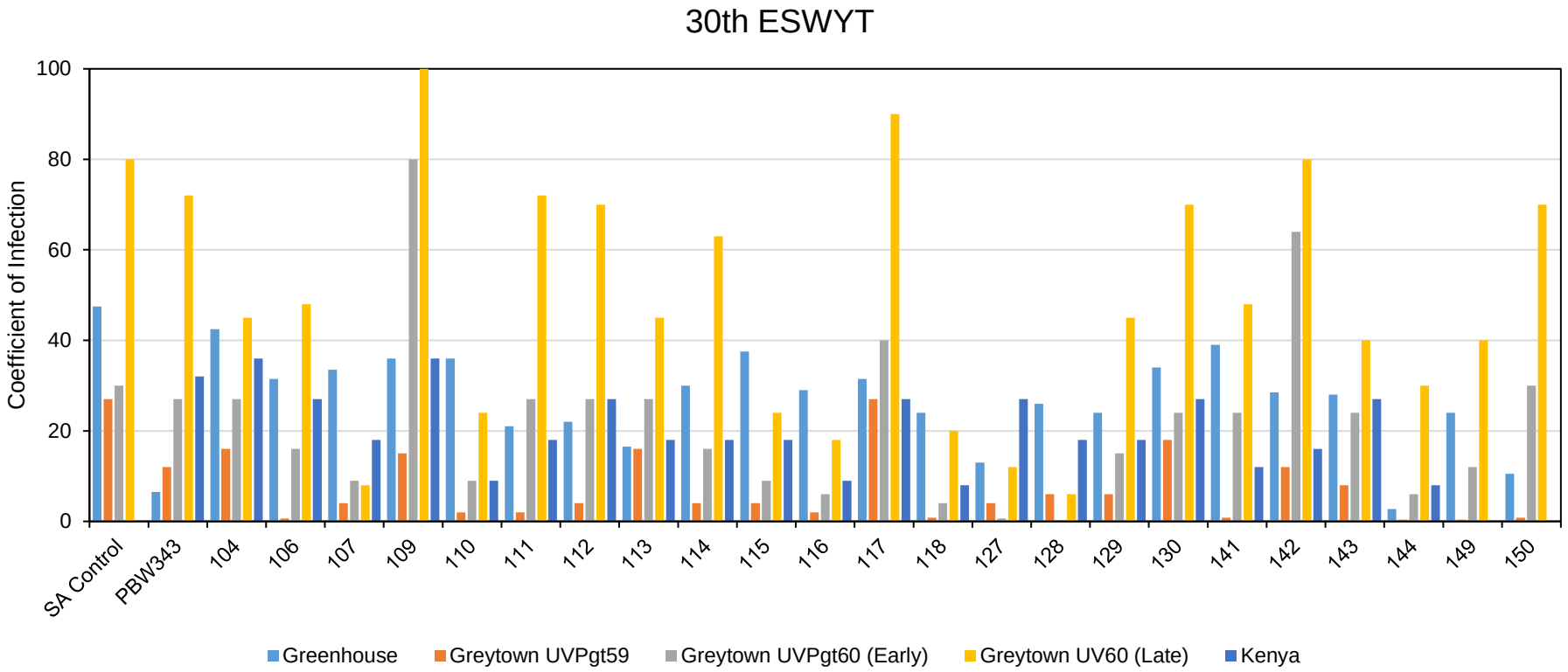

Figure 4.8: Coefficient of Infection calculated for adult plants of 24 30th ESWYT entries infected with urediniospores of *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 in the greenhouse as well as for the first field trial near Greytown. Urediniospores of pathotype UVPgt60 was used to inoculate the second field trial (scored early and late) planted near Greytown and race Ug99 (TTKSK) in Kenya.

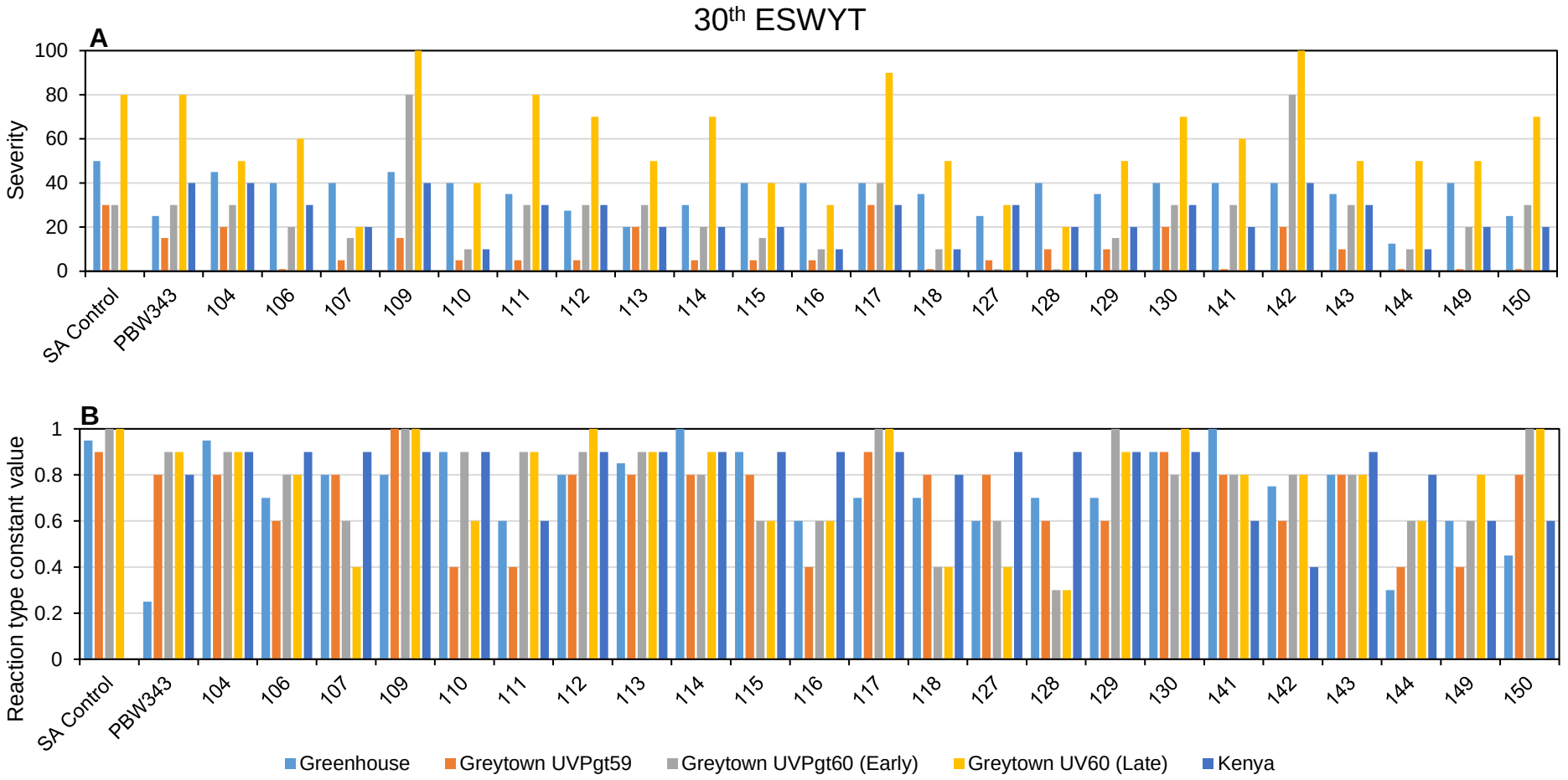

Figure 4.9: Severity (A) and stem rust reaction type (constant value) (B) for adult plants of 24 selected 30th ESWYT entries infected with urediniospores of *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 in the greenhouse as well as for the first field trial near Greytown. Urediniospores of pathotype UVPgt60 was used to inoculate the second field trial (scored early and late) planted near Greytown and race Ug99 (TTKSK) in Kenya.

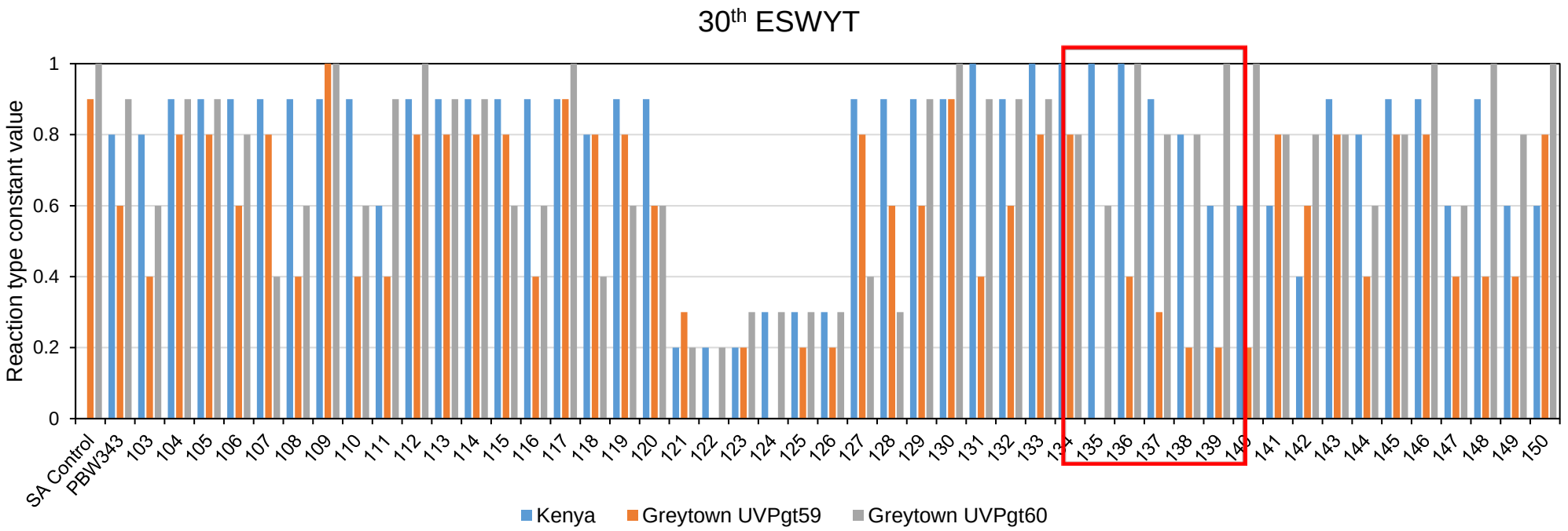

Figure 4.10: Stem rust reaction type (constant value) recorded on adult plants of all 50 trial entries of the 30th ESWYT infected with *Puccinia graminis* f. sp. *tritici* race Ug99 in Kenya. Urediniospores of pathotype UVPgt59 was used to inoculate the first field trial planted near Greytown, KwaZulu-Natal and pathotype UVPgt60 in the second field trial planted near Greytown.

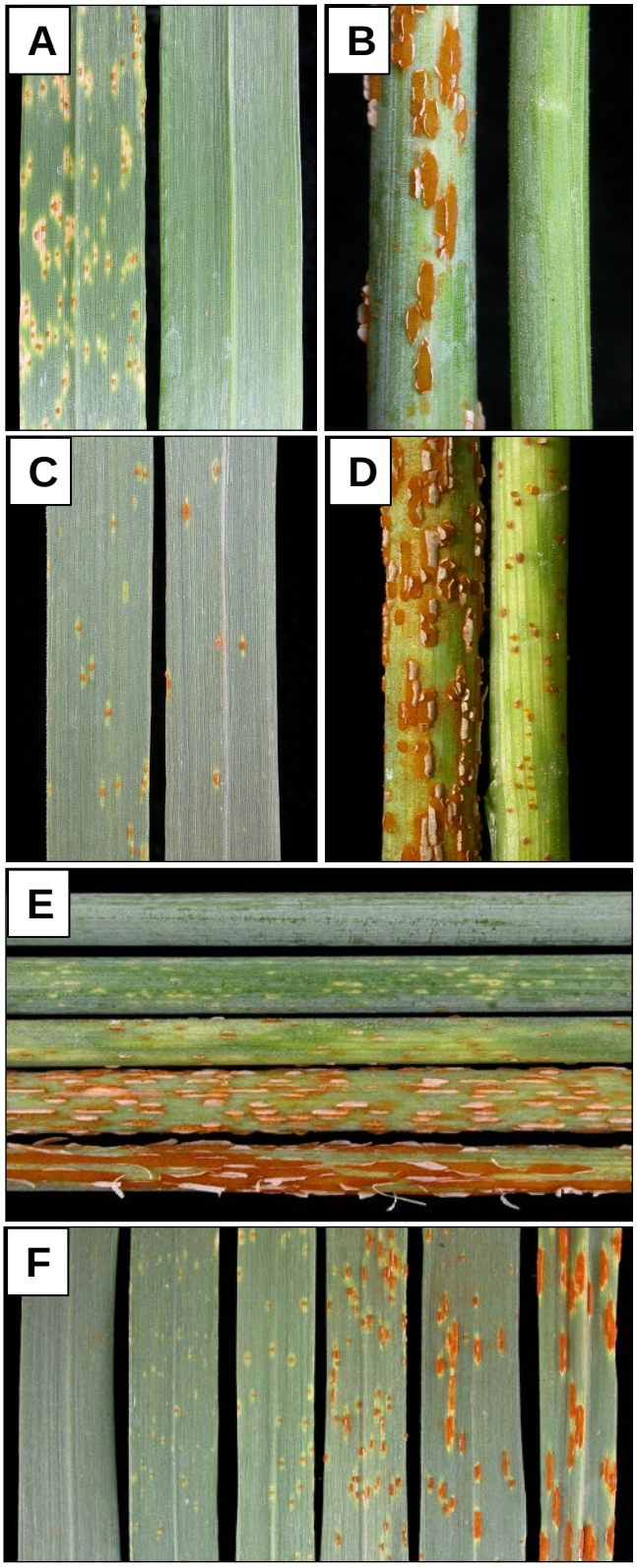

Figure 4.11: Flag leaves and stems of Kariega (left) and Avocet S (right) infected with *Puccinia graminis* f. sp. *tritici* pathotypes UVPgt57 (A and B) and UVPgt59 (C and D) and the infection range on stems (E) of double haploid lines MP21, MP140, MP144, MP112, MP16 (top to bottom) infected with pathotypes UVPgt57 and on flag leaves (F) of MP137, MP17, MP11, MP125, MP108 and MP130 (left to right) infected with pathotype UVPgt59. All pictures were derived from the 1st greenhouse trial replicate.

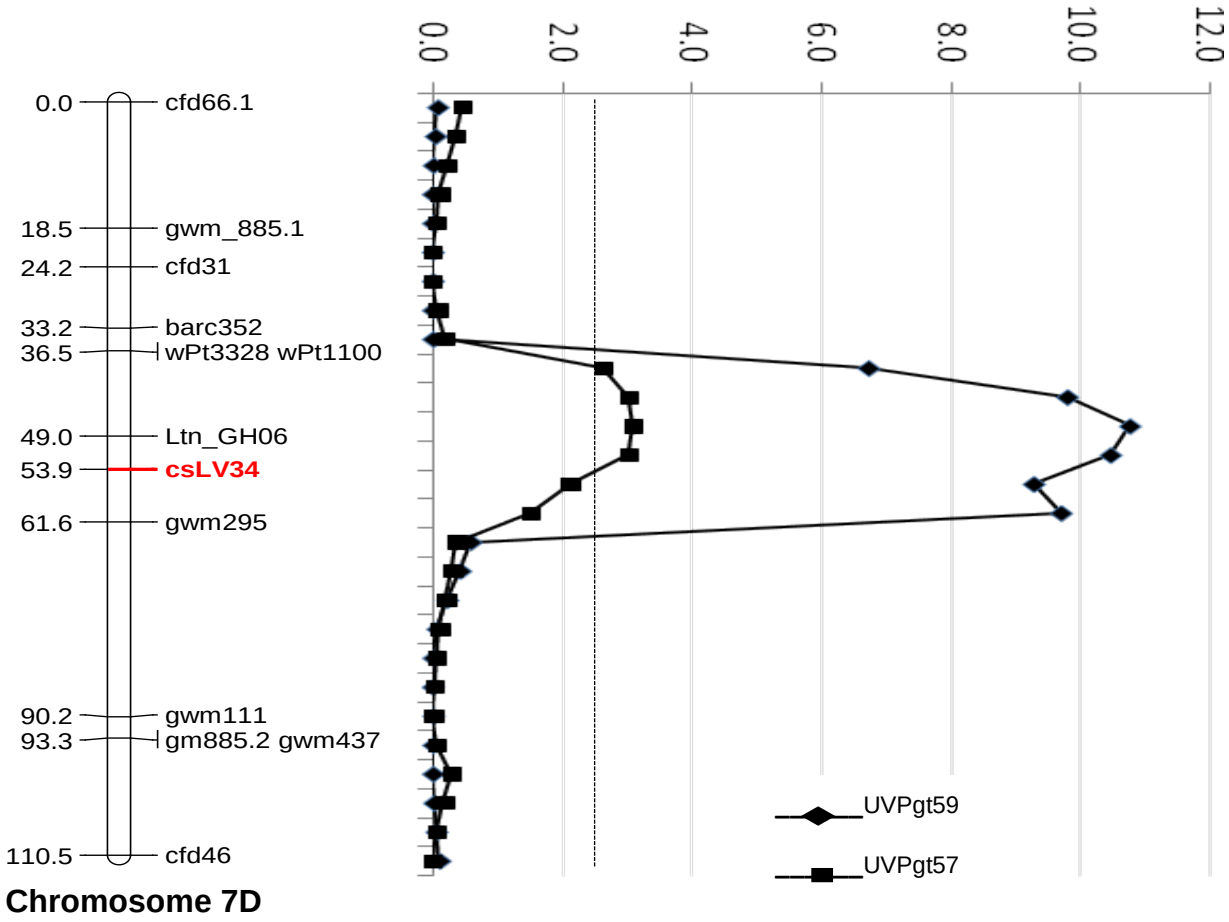

Figure 4.12: Mapping of the *Sr57/Lr34/Yr18* region on chromosome 7D according to flag leaf infection types of 254 Kariega x Avocet S doubled haploid lines inoculated with pathotypes UVPgt57 and UVPgt59 of *Puccinia graminis* f. sp. *tritici* in a greenhouse. Marker loci are shown on the vertical axis. LOD values above 2.5, based on 1000 permutations, were significant at the 5% level. The UVPgt57 dataset was less informative due to avirulence of this pathotype to *Sr5* which also segregated in the population.

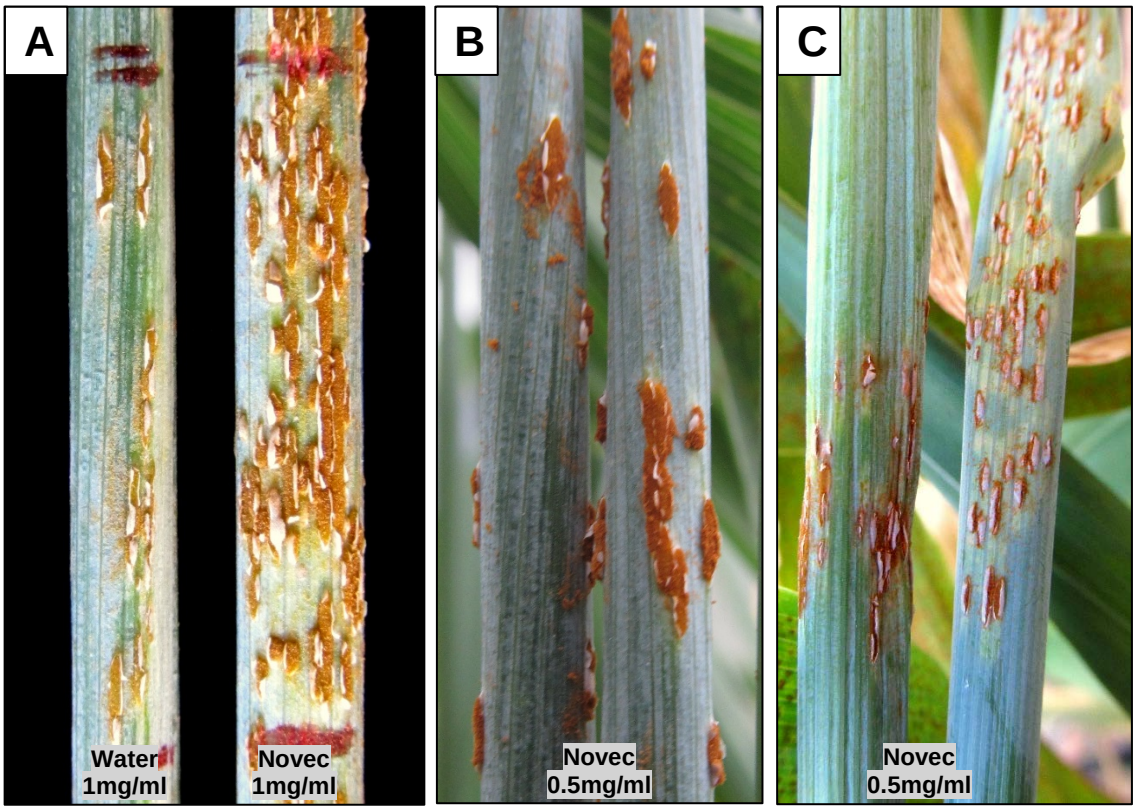

Figure 4.13: Adult plant response to pathotype UVPgt60 on wheat Line 37-07 (A and B) and Gus barley (C) inoculated with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici*. Comparative pictures were taken 14 days after inoculation in the 2nd greenhouse trial. replicate.

Appendix A. Complete list including cross name, selection history and origin of the 30th ESWYT entries

Cooperator ID:	1066
TID :	40807
OCC :	42
Trial Name :	30TH ELITE SPRING WHEAT YT
Trial Abbr:	30ESWYT
Cycle :	INT2009
Program :	BW

Cross Name	Selection History	Origin	Entry
LOCAL CHECK			101
PBW343	CM85836-4Y-0M-0Y-8M-0Y-0IND	MXI07-08\Mult30ESWYT\1	102
WAXWING	CGSS96B00132F-099B-026Y-099M-5Y-0B	MXI07-08\Mult30ESWYT\2	103
PRL/2*PASTOR	CGSS97Y00034M-099TOPB-027Y-099M-099Y-099M-27Y-0B	MXI07-08\Mult30ESWYT\3	104
SERI.1B*2/3/KAUZ*2/BOW//KAUZ	CGSS97Y00030F-099TOPB-059Y-099M-099Y-099M-56Y-0B	MXI07-08\Mult30ESWYT\4	105
WAXWING*2/KIRITATI	CGSS01B00054T-099Y-099M-099M-099Y-099M-13Y-0B	MXI07-08\Mult30ESWYT\95	106
KIRITATI//2*PBW65/2*SERI.1B	CGSS02B00119T-099B-099Y-099M-099Y-099M-39WGY-0B	MXI07-08\Mult30ESWYT\10	107
KIRITATI/4/2*SERI.1B*2/3/KAUZ*2/BOW//KAUZ	CGSS02B00120T-099B-099Y-099M-099Y-099M-18WGY-0B	MXI07-08\Mult30ESWYT\12	108
KIRITATI/2*WBLL1	CGSS02B00131T-099B-099Y-099M-099Y-099M-12WGY-0B	MXI07-08\Mult30ESWYT\15	109
KIRITATI//2*SERI/RAYON	CGSS02B00132T-099B-099Y-099M-099Y-099M-18WGY-0B	MXI07-08\Mult30ESWYT\17	110
WEAVER/TSC//WEAVER/3/WEAVER/4/2*WAXWING	CGSS02B00133T-099B-099Y-099M-099Y-099M-22WGY-0B	MXI07-08\Mult30ESWYT\20	111
WAXWING//PFAU/WEAVER	CGSS03B00144S-099M-099Y-099M-10WGY-0B	MXI07-08\Mult30ESWYT\23	112
HUW234+LR34/PRINIA//PFAU/WEAVER	CGSS03B00148S-099M-099Y-099M-8WGY-0B	MXI07-08\Mult30ESWYT\24	113
PBW343*2/KUKUNA//KIRITATI	CGSS03B00150S-099M-099Y-099M-4WGY-0B	MXI07-08\Mult30ESWYT\26	114
INQALAB 91*2/KUKUNA//KIRITATI	CGSS03B00154S-099M-099Y-099M-5WGY-0B	MXI07-08\Mult30ESWYT\27	115

Appendix A cont. Complete list including cross name, selection history and origin of the 30th ESWYT entries

Cross Name	Selection History	Origin	Entry
SAAR/WAXWING	CGSS03B00162S-099M-099Y-099M-5WGY-0B	MXI07-08\Mult30ESWYT\28	116
SAAR/WBLL1	CGSS03B00166S-099M-099Y-099M-19WGY-0B	MXI07-08\Mult30ESWYT\31	117
SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/KUKUNA	CGSS03B00169S-099M-099Y-099M-32WGY-0B	MXI07-08\Mult30ESWYT\34	118
SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/KUKUNA	CGSS03B00169S-099M-099Y-099M-39WGY-0B	MXI07-08\Mult30ESWYT\35	119
PBW343*2/KUKUNA/5/CNO79//PF70354/MUS/3/PASTOR/4/BAV92	CGSS03B00180S-099M-099Y-099M-20WGY-0B	MXI07-08\Mult30ESWYT\38	120
WHEAR/VIVITSI//WHEAR	CGSS03B00069T-099Y-099M-099Y-099M-34WGY-0B	MXI07-08\Mult30ESWYT\39	121
WHEAR/KUKUNA//WHEAR	CGSS03B00070T-099Y-099M-099Y-099M-50WGY-0B	MXI07-08\Mult30ESWYT\40	122
WHEAR/TUKURU//WHEAR	CGSS03B00074T-099Y-099M-099Y-099M-2WGY-0B	MXI07-08\Mult30ESWYT\42	123
WHEAR/TUKURU//WHEAR	CGSS03B00074T-099Y-099M-099Y-099M-7WGY-0B	MXI07-08\Mult30ESWYT\43	124
WHEAR/KIRITATI/3/C80.1/3*BATAVIA//2*WBLL1	CGSS03B00077T-099Y-099M-099Y-099M-47WGY-0B	MXI07-08\Mult30ESWYT\45	125
WHEAR/VIVITSI/3/C80.1/3*BATAVIA//2*WBLL1	CGSS03B00079T-099Y-099M-099Y-099M-3WGY-0B	MXI07-08\Mult30ESWYT\46	126
WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1	CGSS03B00080T-099Y-099M-099Y-099M-7WGY-0B	MXI07-08\Mult30ESWYT\52	127
WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1	CGSS03B00080T-099Y-099M-099Y-099M-23WGY-0B	MXI07-08\Mult30ESWYT\54	128
WHEAR/KURUKU/3/C80.1/3*BATAVIA//2*WBLL1	CGSS03B00085T-099Y-099M-099Y-099M-27WGY-0B	MXI07-08\Mult30ESWYT\57	129
CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/PRL/2*PASTOR	CMSS02Y00504S-7Y-0M-099Y-3M-0WGY-0B	MXI07-08\Mult30ESWYT\59	130
PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI	CMSS02Y00596S-16Y-0M-099Y-3M-0WGY-0B	MXI07-08\Mult30ESWYT\61	131
PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI	CMSS02Y00596S-34Y-0M-099Y-5M-0WGY-0B	MXI07-08\Mult30ESWYT\62	132
PRL/2*PASTOR/4/CHOIX/STAR/3/HE1/3*CNO79//2*SERI	CMSS02Y00596S-43Y-0M-099Y-2M-0WGY-0B	MXI07-08\Mult30ESWYT\64	133
PFAU/MILAN/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR	CMSS02Y00613S-59Y-0M-099Y-5M-0WGY-0B	MXI07-08\Mult30ESWYT\65	134

Appendix A cont. Complete list including cross name, selection history and origin of the 30th ESWYT entries

Cross Name	Selection History	Origin	Entry
CIRCUS/ELVIRA//PFAU/WEAVER	CMSS02Y02236T-060M-6Y-0M-099Y-3M-0WGY-0B	MXI07-08\Mult30ESWYT\66	135
CHEN/AE.SQ//2*OPATA/3/TILHI/4/ATTILA/2*PASTOR	CMSS02Y02518T-060M-1Y-0M-099Y-5M-0WGY-0B	MXI07-08\Mult30ESWYT\67	136
ELVIRA/5/CNDO/R143//ENTE/MEXI75/3/AE.SQ/4/2*OCI/6/VEE/ PJN// KAUZ/3/PASTOR	CMSS02M01105T-030M-18Y-0M-099Y-4M-0WGY-0B	MXI07-08\Mult30ESWYT\68	137
CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ/6/FRET2	CMSS99M00451S-040M-030Y-030M-11Y-3M-0Y	MXI07-08\Mult30ESWYT\74	138
PFAU/SERI.1B//AMAD/3/WAXWING	CGSS02Y00153S-099M-099Y-099M-46Y-0B	MXI07-08\Mult30ESWYT\78	139
WAXWING*2/VIVITSI	CGSS01B00056T-099Y-099M-099M-099Y-099M-14Y-0B	MXI07-08\Mult30ESWYT\79	140
KIRITATI//PBW65/2*SERI.1B	CGSS02Y00139S-099M-099Y-099M-6Y-0B	MXI07-08\Mult30ESWYT\80	141
BABAX/LR42//BABAX*2/3/VIVITSI	CGSS01B00046T-099Y-099M-099M-099Y-099M-27Y-0B	MXI07-08\Mult30ESWYT\82	142
WAXWING*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	CGSS01B00055T-099Y-099M-099M-099Y-099M-45Y-0B	MXI07-08\Mult30ESWYT\85	143
WAXWING*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ	CGSS01B00055T-099Y-099M-099M-099Y-099M-89Y-0B	MXI07-08\Mult30ESWYT\87	144
WAXWING*2/VIVITSI	CGSS01B00056T-099Y-099M-099M-099Y-099M-26Y-0B	MXI07-08\Mult30ESWYT\88	145
FRET2*2/BRAMBLING	CGSS01B00060T-099Y-099M-099M-099Y-099M-44Y-0B	MXI07-08\Mult30ESWYT\90	146
WBLL1*2/BRAMBLING	CGSS01B00062T-099Y-099M-099M-099Y-099M-47Y-0B	MXI07-08\Mult30ESWYT\91	147
WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-9Y-0B	MXI07-08\Mult30ESWYT\92	148
KIRITATI//SERI/RAYON	CGSS02Y00152S-099M-099Y-099M-46Y-0B	MXI07-08\Mult30ESWYT\93	149
WBLL1*2/KIRITATI	CGSS01B00063T-099Y-099M-099M-099Y-099M-56Y-0B	MXI07-08\Mult30ESWYT\94	150

Chapter 5: Genetics of stem rust resistance in selected South African wheat cultivars

INTRODUCTION

Stem rust, caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn (*Pgt*), is commonly found on wheat (*Triticum aestivum* L.) during rust surveys in South Africa (SA) (Terefe *et al*., 2016). The disease is under control in the more disease-prone production areas through a combination of preventative chemical control and the use of resistant cultivars. Worldwide, resistance to stem rust has often been based on all-stage or race specific seedling resistance (ASR) genes such as *Sr31* (Singh *et al*., 2015). Likewise, the historic deployment of major genes in SA against stem rust has resulted in a narrow genetic base of resistance (Lombard, 1986, Pretorius *et al*., 2012). Many of these single genes have failed due to the evolution of new pathogen races, such as race Ug99 and its 13 variants (Bhavani *et al*., 2019). New Ug99 races detected in SA include UVPgt55 (TTKSF, 2000), UVPgt59 (TTKSP, 2007), UVPgt60 (PTKST, 2009), UVPgt61 (TTKSF+*Sr9h*, 2010) and UVPgt63 PTKSK (2017) (Singh *et al*., 2015, Terefe *et al*., 2019). Other SA races comprise UVPgt50 (PSKSC, 1981), UVPgt52 (PSHSM, 1984), UVPgt53 (BPGSC+*Sr27*, 1988) UVPgt54 (BNGSC, 1964 & 2000), UVPgt54 (BNGSC+*Sr9g*, 2000), UVPgt56 (BPGSC+*Sr27*+*Kw*, 2003), UVPgt57 (BPGSC+*Sr27*+*Kw*+*Satu*, 2005), UVPgt58, (BNGSC+*Sr27*, 1988), and UVPgt62 (BFBSC+*Sr27*, 2010) (Boshoff, 2000, Terefe *et al*., 2016). An increase in virulence remains a constant constraint for genetic control, especially taking into consideration that since the early 1980s, nearly 30 *Pgt* races have been identified in SA (Terefe *et al*., 2016).

To stay ahead of an evolving pathogen population, there is a constant need to discover new sources of resistance, understand the genetic base of presently deployed sources in cultivars and to manipulate the future deployment of resistant sources through a more sustainable approach. Therefore, the purpose of this research was to determine the genetic base of stem rust resistance in selected SA bread wheat cultivars through inheritance studies. Genetic studies including seedling analyses to detect the presence of ASR and field work to detect adult-plant resistance (APR) are essential and can assist breeders to discern between

monogenic and more complex resistance sources. For seedling resistance studies, wheat cultivars displaying low seedling infection types (SITs) in the greenhouse against *Pgt* were selected from the results of Pretorius *et al*. (2008) who screened 54 South African cultivars and 18 breeding lines using races BCCB, MCCF, QFCS, QTHJ, RCRS, RKQQ, TPMK, TTTT, TTKSK and TTKST. Pretorius *et al*. (2012) revealed that 88% of the wheat varieties tested were susceptible to stem rust as seedlings and stated that disease-focused breeding and diversification of resistance sources are required in SA. Furthermore, for APR studies, wheat cultivars displaying high levels of resistance to Ug99-type races in the field were selected.

The use of durable genetic sources to *Pgt* and other wheat rusts has been recommended such as the *Sr2*/*Yr30*, *Lr34*/*Yr18*/*Sr57* and *Lr46*/*Yr29*/*Sr58* complexes in combination with other resistance genes in adult plants to establish more complex resistance in commercial cultivars (Park, 2008, Pretorius *et al*., 2017). The combination of *Sr2* with supplementary genes has contributed to durable stem rust resistance in commercial wheat varieties for almost 100 years (Hare and McIntosh 1979, Njau *et al*., 2010, Singh *et al*., 2008, Sunderwirth and Roelfs 1980). According to Singh *et al*. (2000) combining *Lr34*/*Yr18*/*Sr57* with slow rusting genes can contribute to near immunity to both leaf- and stripe rust. The *Lr46*/*Yr29*/*Sr58* complex, widely distributed in CIMMYT germplasm, confers slow rusting to leaf- and stripe rust (Gupta *et al*., 2017, William *et al*., 2003).

The objective of this study was to analyze the genetics of stem rust resistance in five selected South African spring wheat cultivars namely Duzi, SST 047, Steenbras, Krokodil, and Tankwa as well as the facultative cultivar Betta that was commonly used as a parent in winter wheat breeding in SA against pathotype UVPgt60 (North American race classification PTKST) (Pretorius *et al*., 2012).

MATERIAL AND METHODS

Wheat genotypes

Spring wheat cultivars included in this study were selected based on their resistance expressed to *Pgt* races BCCB, MCCF, QFCS, QTHJ, RCRS, RKQQ,

TPMK, TTTT, TTKSK and TTKST tested by Pretorius *et al*., (2008). Two wheat cultivars (SST 047 and Steenbras) displaying low seedling infection types (SIT = 0;) were chosen for inheritance studies in the greenhouse. Three cultivars (Duzi, Krokodil and Tankwa), displaying high levels of resistance (5MR, 15MR, 15MRMS) to pathotype UVPgt60 in the field, were selected for studying the expression of APR in field trials. Furthermore, the facultative wheat cultivar Betta (20MRMS) (synonym Klein Impacto, of Argentinian origin) was selected based on its common use in resistance breeding in SA and its seedling response to *Pgt* race UVPgt60. The available pedigree information, known genes and stem rust reactions of each parent are provided in Table 5.1. The F2 populations were produced by crossing Duzi, SST 047, Steenbras, Krokodil, Tankwa and Betta with the moderately susceptible cultivar Olifants (typical field response = 50MS) (Pretorius *et al*., 2012) and susceptible Line 37-07 (100S). Line 37-07 originates from the 2nd International Stem Rust Trap Nursery (ISRTN07), entry 37, and was used in this study based on its susceptibility to stem rust in field trials in SA.

Resistant cultivars were used as female parents and the two susceptible lines were the pollen donors. Only Betta was vernalized for 6 weeks at 6°C, but none of its offspring needed low temperatures to reach the reproductive stage. Several crosses were made for each combination to ensure enough F1 seeds. However, only ten F1 seeds originating from a single spike were selected and planted in a 1:1 v/v sterilized soil/peatmoss mixture in 1L pots in the greenhouse, set to 18-25°C night/day schedule. After emergence plants were fertilized twice with Multifeed® water-soluble fertilizer ((19:8:16) NPK plus micronutrients, 2.5g/L water), followed by a water suspension of Wonder® 3:2:1, a slow nitrogen release granular fertilizer, at 7-day intervals for the duration of the trial. Spikes of F1 plants were covered before flowering with glassine crossing bags to prevent out-crossing and the F2 seeds were harvested at maturity. F2 seeds were bulked and used for seedling phenotyping in the greenhouse or as adult plants in field trials. In addition, 120 F2 seeds of each cross were randomly selected and planted in 60 1L pots (2 seeds per pot). Plants were grown and maintained as described for F1's. Upon ripening seed of each plant were individually harvested to test as F3 families.

Stem rust pathotype

Wheat plants were inoculated using freshly collected urediniospores from *Pgt* pathotype UVPgt60 (PTKST) avirulent to the genes *Sr9h, 21, 22, 26, 27, 33, 35, 36, 39, Kiewiet, Satu,* and *Tmp* and virulent on *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 24, 30, 31, 38,* and *McN.* UVPgt60 is a member of the Ug99 race group detected for the first time in SA in 2009 on the winter wheat cultivar SST 356 in a rust nursery planted near Greytown, KwaZulu-Natal (Pretorius *et al*., 2010). Urediniospores were multiplied according to Knott (1989) on seedlings of the selective host Federation*4/Kavkaz (carrying *Sr31*) to ensure race purity. Seeds of the selective host were planted in 10 cm diameter plastic pots filled with Mikskaar® potting substrate MPS2. Seedlings were fertilized one day before inoculation with 50 ml per pot water-soluble Multifeed-Classic (Efekto®, NPK Analysis 19:8:16 (43), concentration 2.5g/L water) and fertilization was repeated after inoculation until termination of trials.

Phenotyping

Seedlings – greenhouse trials

For F2 seedling screening originating from the populations SST047/Line37-07 and Steenbras/Line37-07, approximately 210 seeds per population were planted, whereas 194 families of SST047/Line37-07 and 184 of Steenbras/Line37-07 families were evaluated as F3 seedlings in the greenhouse. For F2 population assessment, 15 seeds were planted per 10 cm plastic pot, whereas 25 seeds of each F3 family were planted per pot. All seedlings were planted in Mikskaar® potting substrate MPS2. Greenhouse temperatures were set to an 18-25°C night/day schedule and plants fertilized with water soluble Multifeed-Classic (Efekto®, NPK Analysis 19:8:16 (43), concentration 2.5 g/L water) twice a week until termination of trials.

F2 populations and F3 families were inoculated with freshly harvested urediniospores at the one and half leaf stage, approximately seven to nine days after planting using a urediniospores suspension of ±3 mg/ml Soltrol® 130, Chevron Phillips, Borger, Texas. Leaves were spray-inoculated using a pressure pump at 25 kPa pressure setting (Vacuubrand® pump - model MZ2) connected to an inoculation

device. Thereafter, seedlings were dried in a growth cabinet, fitted with fluorescent tubes (Eurolux G135, G13 9W T8 LED), at 25°C for about 1 h before incubated in darkness in a dew simulation chamber at 19-23°C and >96% relative humidity for 16 h. Hereafter seedlings were returned to the growth cabinet to dry off for at least 2 h and subsequently transferred and maintained under above described conditions in the greenhouse. Fourteen days after inoculation seedlings were rated according to the 0-4 seedling infection type (SIT) scale, where 0 = immune, ; = flecks, c = chlorotic, or n = necrotic, 1 = minute uredinia, 2 = small to medium sized uredinia, 3 = large uredinia sometimes encircled by slim chlorosis, 4 = large uredinia without any chlorosis, X = random spreading of variable-sized uredinia on a single leaf, Y = ordered spreading of different sized uredinia with larger uredinia at leaf tip and Z = ordered spreading of different sized uredinia with larger uredinia at leaf base (Roelfs *et al*., 1992). Seedling chlorosis, observed as flecking on the second leaf of the seedlings and associated with the infected area (Brown, 1997), were noted in the Steenbras/Line37-07 F3 families.

Adult plants – field trials

Susceptible control entries, parental lines, F2 populations and F3 families were hand planted as 1 m rows with an inter-row spacing of 90 cm in a field trial at PANNAR Research Station, outside Greytown, KwaZulu-Natal, SA during the 1st week of June. For ease of single plant evaluations, seed were space-planted in each row for F2 and F3 assessments. Prior to planting, 250 kg 2:3:4 (38) N-P-K plus 0.5% Zn fertilizer was applied per hectare and weeds were controlled throughout the season. Stem rust spreader rows (mostly a mixture of Line 37-07, Morocco and McNair) were grown orthogonally between blocks and as trial borders of at least two rows. Furthermore, a rust-susceptible entry (either Line 37-07, Morocco or McNair) was included at 10-row intervals in all trials. The trials were irrigated to supplement rainfall during dry spells and to ensure favourable conditions for plant growth and consequent rust development.

Spreader rows were spray inoculated 6 weeks after planting with a concentrated inoculum suspension of *Pgt* urediniospores and Soltrol® 130 oil (±3 mg/ml) to initiate early season infection. Following inoculation plants in selected row sections were

allowed to dry off for approximately 1 h before covering them with plastic sheeting for 12 h overnight. Before securing the plastic cover the soil around inoculated plants was watered to promote high humidity inside the plastic sheeting. This process was repeated until a homogeneous infection was established throughout the nursery. Trials were maintained according to PANNAR's protocol to allow for optimum plant and disease development.

For F2 adult plant evaluation approximately 200 seeds were planted per crossing combination. The number of F3 families per combination varied from 23 to 92. F2 populations derived from the Steenbras/Line37-07 and Steenbras/Olifants crosses were included in the field trials as well as the F3 population of Steenbras/Line37-07. The SST047/Line37-07 F2 populations and F3 families were not assessed in the field trials. Due to slow plant development and for improved comparison rating of the F3 families of the Tankwa/Line37-07 cross was repeated in a 2nd season. Pseudo-black chaff symptoms were noted when expression was observed on either lower stem nodes or on the glumes. Population and family ratings were made at the peak of stem rust epidemic development, when susceptible checks attained severities above 50%. Plant responses recorded for F2 populations and F3 families included severity (percentage stem area infected) (Peterson *et al*., 1948) and reaction type (R, MR, MS and S as well as combinations thereof) (Stubbs *et al*., 1986).

Genotyping

For DNA isolation, 5 seeds of each parental line were planted in 10 cm plastic pots with five accessions per pot. All seedlings were planted and maintained as previously described. Four weeks after planting eight 1-cm leaf segments were sampled in the greenhouse with scissors and tweezers, cleaned between samples with 70% (v/v) ethanol, and placed in 1.5 ml eppendorf tubes kept on ice. Immediately after sampling leaf tissue was freeze-dried using the Lasec Alpha 1-2 LDplus (Osterode am Harz, Germany) for three days and stored at -20˚C. Five dry leaf segments together with two 5 mm stainless steel ball were placed in a 2 ml micro centrifuge tube and ground to a fine powder using Qiagen's TissueLyser (Haan, Germany) for 1 min at 30 r/s. For the extraction of genomic DNA (gDNA) the

modified hexadecyltrimethylammonium bromide (CTAB) method of Saghai-Maroof *et al*. (1984) was used. To liquefy the dry plant tissue 750 µl CTAB buffer [100 mM Tris-HCl [tris(hydroxymethyl) aminomethane hydrochloride], pH 8.0; 20 mM EDTA (ethylene-diaminetetraacetate) pH 8.0; 1.4 M NaCl (sodium chloride); 2% (w/v) CTAB; 0.2% (v/v) β-mercaptoethanol] was added to each sample, incubated at 65°C and lightly shaken at 15 min intervals. After incubation 500 µl chloroform:isoamylalcohol [24:1 (v/v)] was added to each sample, mixed and centrifuged for 5 min at 12 000 *g* at 4°C. Thereafter, the supernatant was transferred to a 1.5 ml micro centrifuge tube containing 500 µl (0.66 volumes) 2-isopropanol. DNA was precipitated for 20 min at room temperature (22˚C) followed by centrifugation for 5 min at 12 000 *g* at 4˚C. Supernatant was extracted and discarded and the DNA pellet washed with 500 µl ice cold 70% (v/v) ethanol. Samples were kept at room temperature (22°C) for 20 min, before centrifuged for 5 min at 12 000 *g* at 4˚C. Again, supernatant was discarded before the DNA pellet was air-dried for 1 h at 22°C whereafter it was resuspended in 200 µl TE (Tris-HCl/EDTA) buffer (10 mM Tris-HCI, pH 8.0; 1 mM EDTA, pH 8.0) and 200 µl/ml RNase and incubated at 37°C in a waterbath for 2 h.

Determination of the quality of the gDNA was done on a 0.8% (w/v) agarose gel and ethidium bromide (EtBr) used for visualization with ultraviolet light. Electrophoresis was done at 100 V for 30 min in 1x UNTAN buffer (40 mM Tris-HCl, pH 8.0; 2 mM EDTA pH 8.0; pH was adjusted to pH 7.4 with acetic acid). The Jenway 7315 UV/Visible spectrophotometer, absorbance at A_{260}, were used to quantify DNA, whereafter the concentration was adjusted to 20 ng/µl using 1 x TE buffer, pH 8.0.

The DYAD™ (DNA Engine) Peltier Thermal Cycler (Foster City, CA, USA) was used for PCR reactions set up to a final volume of 10 µl, except when 20 µl was used for the CAPS marker csSr2. Molecular markers linked to specific *Sr* genes were used to genotype the selected cultivars (maswheat.ucdavis.edu /protocols/StemRust/index.htm). Primers synthesised by Intergrated DNA Technologies Inc (Coralville, IA, USA) were used and reaction conditions thereof presented in Table 5.2.

Excluding markers cssfr5 and Iag95, polyacrylamide gel electrophoresis was used to visualize PCR reactions with a GelScan 3000 Real-Time DNA Fragment Analysis system with software version 8.00.01 (Corbett Research, Sydney, Australia). A 5% (w/v) non-denaturing polyacrylamide gel was prepared to a final volume of 25 ml consisting of 1 x TBE (Tris-HCl/Borate/EDTA) buffer (89 mM Trisbase; 89 mM Boric acid; 2 mM EDTA, pH 8.0), 5% AKA (acrylamide:bis-acrylmide; 19:1 w/w), 0.12% (v/v) TEMED (N,N,N',N-tetramethylethylenediamine) and 0.08% (v/v) APS (ammonium persulfate) and kept overnight at room temperature (22°C) to polymerise. The vertical gel system consisted of an upper and bottom chamber, where the upper chamber contained 0.5x TBE buffer mixed with doubled distilled water (ddH_2O) and the bottom buffer contained 0.5x TBE and 1% (v/v) EtBr mixed with ddH_2O. Deionised formamide loading dye [98% (v/v) formamide; 10 mM EDTA, pH 8.0; 0.05% (w/v) bromophenol blue were added to PCR products and depending on signal strength of reactions the loaded volume were adjusted. To track progress a pre-run of 1 µl loading dye was done at 800 V for 45 min at 37˚C. Thereafter, 1 µl of the PCR samples with dye was run at 1 200 V for 45 min at 37˚C. For determination of amplified fragment sizes the 25 bp HyperLadder™V ladder (Bioline, Taunton, MA, USA) flanked both sides of the gel. The PCR products of the excluded markers cssfr5 and Iag95 were separated on a 1.5% (w/v) and 1% (w/v) agarose gel, respectively. Ficoll loading buffer [15% (w/v) Ficoll, 0.24% (w/v) bromophenol blue] were added to these and gels were run at 100 V for 30 min in 1x UNTAN buffer. For determination of fragment sizes a 100bp DNA ladder (Promega, Madison, WI, USA) was used.

Data analysis was based on the type of molecular marker used and samples scored. The amplified fragment of dominant markers when present scored (1) or absent (0). Co-dominant markers indicated homozygous resistant (1), heterozygous resistant (0.5) or homozygous susceptible (0).

RESULTS

Phenotyping

Seedlings – greenhouse trials

Steenbras and SST 047 were resistant to stem rust pathotype UVPgt60 with both expressing a SIT of 0; whereas, Line 37-07 was susceptible with an SIT of 3+ to 4 (Fig 5.1 and 2 A) (Table 5.1). F2 seedlings from the Steenbras/Line37-07 population segregated with typical SITs that varied from 0;, ;1, X to 3+ (Fig 5.1 B). The F2 population from the cross SST047/Line 37-07 varied from 0;, 1, 2 to 3+ (Fig 5.2 B). Evaluations of F2 seedlings with pathotype UVPgt60 from the cross Steenbras/Line37-07 ($\chi_{3:1}$ = 4.174, P = 0.041) indicated the presence of one *Sr* gene, whereas the offspring from the cross SST047/Line37-07 ($\chi_{15:1}$ = 2.091, P = 0.148) segregated for two stem rust resistance genes (Table 5.3).

The evaluation of the F3 seedlings as families more confidently indicated the presence of two seedling genes in the cultivar Steenbras ($\chi_{7:8:1}$ = 4.022, P = 0.134), contradicting the F2 data. The F2 data results for SST 047 was confirmed in the F3 segregation ratios ($\chi_{7:8:1}$ = 3.114, P = 0.211) (Table 5.4). The homozygous resistant F3 families from the cross Steenbras/Line37-07 varied in SIT from 0;, ;1 and 0;, ;1, to X2 (Fig 5.3 A and B), whereas the segregating families always included clearly susceptible seedlings (SITs = 3+ and 4) (Fig 5.4). The F3 families from the cross SST047/Line37-07 revealed similar SITs for seedlings in the resistant families except for an X instead of an X2 (Fig 5.5), while pustules observed on seedlings from segregating F3 families were more fully compatible with typical SITs of 3++ (Fig 5.6). The presence of a gene assumed to be *Sr2* was confirmed in F3 families from the cross Steenbras/Line37-07 in the seedling stage by noting the dominant expression ($\chi_{3:1}$ = 0.307, P = 1.034) of the linked seedling chlorosis gene (Fig 5.7) with 132 of the 184 families that expressed chlorosis.

Adult plants – field trials

Disease pressure observed in the field was adequate to discriminate among stem rust phenotypes of the F2 populations except for Duzi crosses where it was not possible to confidently distinguish between intermediate and susceptible individuals. Adult plant responses of the resistant parental lines varied in the field

from 0R to 20MRMS (Table 5.1) and the resistant responses of Tankwa, Krokodil, Steenbras, Betta are demonstrated in Fig 5.9 A and Duzi Fig 5.9 B, with the susceptible responses of Olifants and Line 37-07 in Fig 5.9 C.

The field trial evaluation of adult F2 plants for Steenbras crossed with either Line 37-07 ($\chi_{15:1}$ = 0.319, P = 0.572) or Olifants ($\chi_{15:1}$ = 0.304, P = 0.581) indicated two major *Sr* genes. This included the presence of *Sr2* which was visible through the expression of stem melanism (Fig 5.8). Both of the Steenbras F2 populations were incorporated in the field trials because of inconsistent greenhouse results and Steenbras/Line37-07 ($\chi_{15:1}$ = 0.319, P = 0.572) and Steenbras/Olifants ($\chi_{15:1}$ = 0.304, P = 0.581) segregation ratios confirmed two genes (Fig 5.10 A&B). The F2 population of Tankwa segregated (Fig 5.10 C and D) for two genes in both crosses, viz. Tankwa/Line37-07 ($\chi_{15:1}$ = 0.133, P = 0.715) and Tankwa/Olifants ($\chi_{15:1}$ = 0.433, P = 0.51).

Segregation of resistance for both F2 populations of Krokodil and Betta fit the model for a single dominant stem rust resistance gene (Fig 5.11). Krokodil/Line37-07 ($\chi_{3:1}$ = 0.069, P = 0.793) and Krokodil/Olifants ($\chi_{3:1}$ = 1.357, P = 0.244) as well as Betta/Line37-07 ($\chi_{3:1}$ = 1.325, P = 0.25) and Betta/Olifants ($\chi_{3:1}$ = 0.144, P = 0.704) segregated for only one *Sr* gene. Segregation of resistance in all F2 populations and their expected ratios are illustrated in Table 5.5.

The evaluation of the F3 families as adult plants in the field trial (Table 5.6) confirmed the hypothesis of two major genes for Steenbras and Tankwa. Similar results were obtained for the cross Duzi/Line37-07 for which segregation ratios were not determined in the F2 population. The expected segregation ratio for F3 families, carrying two genes, namely 7 homozygous resistant : 8 segregating : 1 homozygous susceptible was in agreement with the 68 resistant : 78 segregating : 8 susceptible observed among the Steenbras/Line37-07 families ($\chi_{7:8:1}$ = 0.293, P = 0.863) (Fig 5.12 A). Further screening of the families of Duzi/Line37-07 ($\chi_{7:8:1}$ = 0567, P = 0.729) and Tankwa/Line37-07 ($\chi_{7:8:1}$ = 3.277, P = 0.192) suggested that two genes control resistance to pathotype UVPgt60 (Fig 5.12 C and D). Notable was that the resistant plants in offspring of both Tankwa crosses expressed more

resistant responses, compared to Tankwa (15MRMS). This was true for both F2 and F3 populations where responses varied from 0R, TR to 5R.

The F3 families of both Krokodil/Line37-07 ($\chi_{1:2:1}$ = 1.390, P= 0.499) and Betta/Line37-07 ($\chi_{1:2:1}$ = 3.268, P = 0.195) conformed to the hypothesis of segregation for a single dominant gene in each of the resistant cultivars (Fig 5.13).

Genotyping

The five resistant and two susceptible varieties were successfully evaluated, together with three added varieties namely CnsSrTmp, SST 44, Agent and a Betta R (selected from previously used seed source), with the selected *Sr* markers (Table 5.7). Highlighted in grey (Table 5.7) are the seven control lines included for marker analysis. One of the markers linked to *SrTmp*, Gpw5182, successfully amplified but results were amiss as most of the cultivars showed the same fragment compared to the positive control. Only Steenbras indicated heterozygous resistance with marker Cssfr5 linked to *Sr57* (Figure 5.14).

DISCUSSION

The most sustainable approach to stem rust control is growing of resistant cultivars and numerous studies have been conducted to understand the genetics of resistance expressed at different growth stages (e.g. Dodds and Rathjen, 2010, Lagudah, 2011, Singh *et al*., 2014). According to Ellis *et al*. (2014) major gene or ASR, can be detected in wheat seedlings and generally throughout the following growth stages, whereas APR is best expressed in more mature plants in the field. Single APR genes can provide partial resistance against a range of stem rust pathotypes, but is considered not adequate under conditions of high disease pressure. Therefore, gene stacking of more than one APR gene has been recommended.

Initially genetic control of stem rust was achieved in the more disease prone areas through the deployment of single genes in cultivars, but inevitably, these genes failed due to pathogenicity changes in the *Pgt* population. Pyramiding of several resistance genes has become an important objective in breeding programs and

molecular marker assisted selection is an important aid to achieve gene stacking (Pretorius *et al*., 2017). In addition, phenotyping at the seedling stage under greenhouse conditions allows for screening of many wheat lines in a short period of time and small space compared with adult-plant tests in field trials (Letta *et al*., 2014). Results from this study showed that both stem rust seedling tests in the greenhouse and adult plant screening in the field can provide reliable expression of unidentified resistance genes. This allowed for the accurate prediction of the number of resistance genes involved in the cultivars.

The present study determined the inheritance of resistance in five South African wheat cultivars to pathotype UVPgt60 (member of Ug99 race group). The detection of the Ug99 race (TTKSK) in Uganda in 1999 (Pretorius *et al*., 2000) generated interest globally, because of its virulence to *Sr31*, a widely deployed stem rust resistance gene in Africa and Asia. Since then 13 new variants (Jin *et al*., 2008, 2009, Singh *et al*., 2015, Terefe *et al*., 2019) in the Ug99 lineage were described. Two of these (TTKST and TTTSK) are of particular importance for this study where resistance genes *Sr24* (Jin *et al*., 2008) and *Sr36* (Jin *et al*., 2009) became ineffective. According to Le Roux and Rijkenberg (1987) wheat breeders during the 1980s relied on only a few resistance genes namely *Sr9e*, *Sr24, Sr31* and *Sr36* in the development of commercial wheat cultivars in SA. However, more recently, pedigree information of newly released wheat cultivars remains confidential in SA which prevents any prediction of resistance genes involved based on historic data available for the parents used in the development of these cultivars. Results from recent mapping studies revealed the presence of *Sr9h* (ineffective to *Pgt* TTKSF+ in the Ug99 lineage) in the winter wheat cultivar Matlabas (Wessels *et al*., 2019) as well as an ASR gene on chromosome 6D in the winter wheat cultivars Komati, Koonap, Limpopo, and SST 387 (Chemonges *et al*., 2019), further supporting what is most likely an unintentional deployment of single resistance genes by wheat breeders.

The ASR gene *Sr24*, that originates from *Agropyron elongatum* (syn. *Thinopyrum ponticum*), provided resistance in both seedling and adult plants to stem rust worldwide (Lombard, 1986, Martens, 1985, McIntosh *et al*., 1977, Roelfs *et al*.,

1983). Because of its widespread effectiveness and its close linkage with the leaf rust resistance gene *Lr24* (McIntosh *et al*., 1977), it was commonly used in Australia, United States and in SA, where nine of the 23 cultivars evaluated by Le Roux and Rijkenberg (1987) carried *Sr24*, either alone or in combination with other genes. Increased virulence for *Sr24* was first observed in 1984 by Le Roux and Rijkenberg (1987), but *Sr24* virulence in the Ug99 lineage in SA was only identified in 2007 in the Western Cape (Terefe *et al*., 2010). Despite virulence for *Sr24* in TTKSP, results from recent stem rust surveys showed that the dominating race TTKSF detected during surveys lacks virulence for this gene (Terefe *et al*., 2016; Terefe *et al*., 2019). Previously *Sr24* was confirmed in Duzi (Kariega/Palmiet) (Smit *et al*., 2010), possibly transferred from Palmiet known to carry both *Sr2* and *Sr24* (Pretorius *et al*., 2012), whereas in this study two unidentified genes were expressed under field conditions. Molecular marker csSr2 linked to *Sr2* used in this study confirmed the presence of *Sr24* in Duzi but excluded *SrTmp* as one of the unidentified genes. The pedigree of Palmiet includes both Scout (*Sr2+9d+17+Tmp*) and Agent (*Sr9d+12+24*) whereas, Agent and Anza (*Sr5+8a+9b+12*) were used to develop Kariega (Wheatpedigree.net accessed 01 October 2019). Prins *et al*. (2011) identified the APR *Lr34*/*Yr18*/*Sr57* gene complex in Kariega on chromosome 7D and marker Cssfr5 resolved the identity when it verified *Sr57* as one of the unidentified resistance genes.

The stem rust resistant cultivar Palmiet (*Sr2+24*), released in 1985, was used in the development of Krokodil (Marico*2//PI262660/5*Palmiet) (Smit *et al*., 2010). Other parents in the pedigree of Krokodil, with stem rust resistance genes, include Agent (*Sr24*) and Scout (*Sr9d+17*) in Palmiet and Clement (*Sr31*) in Marico (McIntosh *et al*., 1977, 1995). Using markers, however, Pretorius *et al*. (2012) showed that *Sr31* does not occur in Marico. Krokodil remained moderately resistant to pathotype UVPgt60 in the field trial although all these genes except *Sr2* are ineffective to UVPgt60. Results of this study predict a single adult plant stem rust resistance gene to occur in Krokodil. Although *Sr2* is still effective, its presence in Krokodil could not be confirmed through the expression of melanism in the field or the use of molecular marker csSr2 in this study. This is in support with the findings of a previous study

which indicated the absence of *Sr2* in Krokodil using seedling chlorosis and the csSr2 marker as criteria (Pretorius *et al*., 2012).

Furthermore, the presence of a single dominant stem rust resistance gene was confirmed to occur in Betta. Although no obvious relationship was found between Betta and Triumph (*SrTmp*) in pedigree when searching the IWIS™ database (Payne *et al*., 2002) the presence of *SrTmp* in South African winter wheats cannot be excluded considering the higher SITs obtained with *Pgt* race TTTTF on the cultivars Caledon, Elands, Gariep and Limpopo (Pretorius *et al*., 2012). Betta is considered to be the most likely source of this resistance as it was used as a parent in all of these cultivars (Boshoff, 2000, Smit *et al*., 2010). Lombard (1986) identified *Sr8a* in four derivatives of Betta and postulated the presence of *Sr2* in Betta, but Le Roux and Rijkenberg (1989), identified the gene as a different stem rust resistance gene than *Sr2*. Molecular markers confirmed the presence of *Sr24* in the Betta female plant used for crosses in this study, but exclude *SrTmp* as the unidentified gene. However, more recent seedling tests with a Betta seed source obtained from Dr Vicki Tolmay, Agricultural Research Council-Small Grain, revealed segregation in resistance to race PTKST. Results obtained with the Barc183 marker confirmed these “Betta R” seedlings positive for the *SrTmp* gene and negative for *Sr24* (Sr24#50). This indicate that Betta may segregate for both *Sr24* and *SrTmp* or that one of the two Betta seed sources are at fault.

Sr2 is possibly the best known wheat stem rust resistance gene and has provided long lasting resistance since its transfer from tetraploid emmer wheat (*Triticum dicoccum* Schronk) in the 1920’s. (McFadden, 1930). *Sr2* resistance is expressed only in the adult plant stage against all known *Pgt* pathotypes, including the Ug99 race group, but when singly deployed under epidemic conditions it fails to provide adequate protection due to its partial nature (Mago *et al*., 2011). Dark pigmentation or necrotic areas on the peduncles and glumes, known as pseudo black chaff (PBC), and pigmentation on stems are associated with *Sr2* resistance (Kota *et al*., 2006). Although breeders sometimes rely on this trait to detect the presence of *Sr2* in field grown adult plants, it is not always considered as a reliable indicator due to variation in expression of pigmentation levels that can occur depending on the

prevailing environmental conditions, the genotype involved, as well as the higher tendency of expression towards the end of the season (Mago *et al*., 2011). *Sr2* was confirmed by Pretorius *et al*. (2012) in Steenbras and was probably transferred from an old SA cultivar Hoopvol occurring in the pedigree of this cultivar. The seedling gene *Sr36*, together with an unidentified resistance gene, was postulated to occur in Steenbras as it produced a SIT of 2 to *Pgt* race TTTTF in a previous study (Pretorius *et al*., 2012). This response clearly differed from the typical SIT of 0; commonly associated with the presence of *Sr36* (McIntosh *et al*., 1995) produced by the races TTKSK, TTKST, TTKSF, TTKSP, and PTKST on *Sr36* included in the latter study. The presence of *Sr2* was confirmed with the molecular marker csSr2. However, Steenbras marked negative for the presence of *Sr36* using Gwm319. This negative result was confirmed in an independent repeat. However, the unexpected heterozygous expression of the *Sr57* gene with the Cssfr5 molecular marker, possibly transferred from SST 44 that tested positive for both *Sr24* and *Sr57*, could probably explain the inconsistent results when used as a crossing parent. Sunderwirth and Roelfs (1980) concluded that anthesis is the earliest growth stage for reliable expression of *Sr2*. Therefore, the discrepancy between the F2 and F3 populations for the cross Steenbras/Line37-07 in the greenhouse can furthermore perhaps be ascribed to the partial resistance phenotype of *Sr2* masked by the other resistance gene. Furthermore, Knott (1989) stated that *Sr2* also act as a modifier of seedling resistance. However the expression of *Sr2* in the F3 families of the cross Steenbras/Line37-07 in the greenhouse as seedling chlorosis was distinctive and consistent with previous data suggesting a single gene action (Brown, 1997). These data support the routine screening of lines for *Sr2* in the greenhouse based on seedling chlorosis.

Two major stem rust resistance genes were expressed in Tankwa, currently classified as moderately susceptible to both stem- and leaf rust and resistant to stripe rust in the production guidelines of the Agricultural Research Council Small-Grain (ARC-Small-Grain, 2019). However, field results recorded in this study for Tankwa (15MRMS), indicate that a moderately resistant classification would be more appropriate. The segregation of both F2 and F3 populations was clear with

clear resistance responses. Although *Sr31* was confirmed in Tankwa with the lag95 molecular marker, the other two genes are still unidentified.

Previous data postulated two resistance gene complexes *Sr24*/*Lr24* and *Sr38*/*Lr37*/*Yr17* (De Groot, 2012) and *Sr36* (Prins *et al*., 2016) in SST 047. *Sr36*, derived from *Triticum timopheevii* (Allard and Shands, 1954), conferred a near immune resistance reaction to Ug99 until 2007 when virulence for this gene was discovered in Kenya (Jin *et al*., 2009). The 0R response type recorded with stem rust pathotype UVPgt60 for SST 047 in the field supported the plausibility of *Sr36* and the current molecular study confirmed the presence of *Sr24* and *Sr36* with markers Sr24#50 and Gwm319 respectively. Furthermore low SIT were reported by Newcomb *et al*. (2016) on entries carrying *Sr36* with Ug99 races TTKSK, TTKST, TTKTK, TTKTT and TTHST similar to the low SIT of PTKST on SST 047. In a study with *SrTt-1* (synonym *Sr36*) Rowell (1981), in agreement with this study, documented X SIT in some of the progenies and the low SIT recorded on *SrTt-1* by Roelfs and McVey (1979) varied from 0 to X^{-}. Previous studies did report heterogenous SIT of 0;3 (Knott, 1990) and 0;4 (Roelfs and McVey, 1979) expressed by *SrTt-1*.

Virulence for *Sr36* was documented in SA in 1964 (Lombard and Lombard, 1965), but subsequently disappeared until 1981 despite the production of cultivars carrying this gene (Le Roux, 1986). According to Le Roux (1986) widespread drought conditions that prevailed during 1982 and 1983 might have contributed to the absence of stem rust during surveys conducted during these years. Thereafter 2SA36 virulent to *Sr36* was detected during 1993 with 10.2% of the pathotypes detected during surveys that typed as this race (Boshoff *et al*., 2000). Virulence for *Sr36* has not been detected in SA since then (Terefe *et al*., 2010, 2016).

Studies to determine the genetic base of stem rust resistance were informative in determining the complexity of resistance sources deployed. The stacking of resistance genes as observed for the cultivars Duzi, SST 047, Steenbras and Tankwa as well as unidentified APR that occurs in the cultivar Krokodil should be recommended. The results further confirm the presence of monogenic resistance,

vulnerable to pathogenic adaption, in the winter wheat Betta which is probably the same source of stem rust resistance that occurs in a number of winter wheat cultivars in SA. The results from this study should help breeders to distinguish between complex and monogenic resistance sources and facilitate their use in deploying more complex resistance combinations.

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Table 5.1: Wheat parents used in crosses, their status, pedigree, origin, seedling infection types (SIT) and adult plant response (APR) to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Parents	Status	Pedigree	Origin	SIT[a]	APR[b]
Steenbras	Cultivar	SST44/SST66/4/Hoopvol/CI296001/3/T.aestivum/Bonanza//Ciano/7C	South Africa	0;	TR
SST 047	Cultivar	Confidential	South Africa	0;	0R
Duzi	Cultivar	Kariega/Palmiet	South Africa	11+	5MR
Tankwa	Cultivar	Confidential	South Africa	2+	15MRMS
Krokodil	Cultivar	Marico*2//PI262660/5*Palmiet	South Africa	4	15MR
Betta	Cultivar	Lee/Frontana Klein Lucero/Klein 157//Klein 157/3/Klein Orgullo	Argentina	3++	20MRMS
Olifants*	Cultivar	Jupateco'S'/Bobwhite'S'//Veery#5/Buckbuck'S'/3/Tui'S'	South Africa	4	50MS
Line 37-07*	Line	Kasyob/Genaro-81//Cham4	ISRTN07 Mexico	4	60S

[a] Seedling infection types (SIT) were determined 14 days after inoculation
[b] Adult plant response were determined under field conditions in a stem rust nursery planted near Greytown KwaZulu-Natal
* Susceptible parents used in the crosses

Table 5.2: Selected molecular markers used to evaluate cultivars reaction conditions and annealing temperatures.

Marker name	Targeted *Sr* gene	Positive control	PCR conditions	PCR annealing temperature (ºC)
csSr2	*Sr2*	Kingbird	100 ng gDNA 1 x TEMPase Hot Start Master mix 50 ng Forward Primer 50 ng Reverse Primer	56.7
Sr24#50	*Sr24*	Palmiet	60 ng gDNA 1 x TEMPase Hot Start Master mix 50 ng Forward Primer 50 ng Reverse Primer	65
Iag95	*Sr31*	Federation4*/Kavkaz	80 ng gDNA 1 x TEMPase Hot Start Master mix 25 ng Forward Primer 25 ng Reverse Primer	50
Gwm319	*Sr36*	W2691SrTt-1 (W3528)	80 ng gDNA 1 x TEMPase Hot Start Master mix 25 ng Forward Primer 25 ng Reverse Primer	55
Gpw5182	*SrTmp*	Cns-SrTmp	80 ng gDNA 1 x TEMPase Hot Start Master mix 25 ng Forward Primer 25 ng Reverse Primer	60
Barc183	*SrTmp*	Cns-SrTmp	80 ng gDNA 1 x TEMPase Hot Start Master mix 25 ng Forward Primer 25 ng Reverse Primer	60
Cssfr5	*Sr57*	Kariega	60 ng gDNA 1 x TEMPase Hot Start Master mix 50 ng Forward Primer 50 ng Reverse Primer	58

PCR - Polymerase chain reaction, *Sr* - stem rust

Table 5.3: Segregation ratios of F2 populations determined in the greenhouse on seedlings inoculated with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Cross	Resistant	Intermediate	Susceptible	R:S[a] ratio	$\chi 2$ [b]	*P* value
Steenbras/Line37-07	86	64	34	3:1	4.174	0.041
SST047/Line37-07	106	71	17	15:1	2.091	0.148

[a] R=resistant and intermediate, S=susceptible
[b] Significant limit of $\chi 2$ (p = 0.05, df = 1)
Seedling infection types were determined 14 days after inoculation

Table 5.4: Segregation ratios of F3 families determined in the greenhouse on seedlings inoculated with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Cross	Resistant	Segregating	Susceptible	R:Seg:S[a] ratio	$\chi 2$ [b]	*P* value
Steenbras/Line37-07	90	79	15	7:8:1	4.022	0.134
SST047/Line37-07	84	92	18	7:8:1	3.11362	0.211

[a] R=resistant, Seg=segregating, S=susceptible
[b] Significant limit of $\chi 2$ (p = 0.05, df = 2)
Seedling infection types were determined 14 days after inoculation

Table 5.5: Segregation ratios of F2 populations determined in the adult plant stage in a field trial planted near Greytown, KwaZulu-Natal to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Cross	Resistant	Intermediate	Susceptible	R:S[a] ratio	$\chi 2$ [b]	*P* value
Steenbras/Line37-07	114	38	12	15:1	0.319	0.572
Steenbras/Olifants	106	57	9	15:1	0.304	0.581
Tankwa/Line37-07	95	58	9	15:1	0.133	0.715
Tankwa/Olifants	106	63	9	15:1	0.433	0.51
Krokodil/Line37-07	132		42	3:1	0.069	0.793
Krokodil/Olifants	131		35	3:1	1.357	0.244
Betta/Line37-07	134		36	3:1	1.325	0.25
Betta/Olifants	138		49	3:1	0.144	0.704

[a] R=resistant and intermediate, S=susceptible

[b] Significant limit of $\chi 2$ ($p = 0.05$, $df = 1$)

Adult plant response ratios were determined at the peak of stem rust season (Line 37-07 > 60S and Olifants > 50MS))

Table 5.6: Segregation ratios of F3 families determined in the adult plant stage in a field trial planted near Greytown, KwaZulu-Natal to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Cross	Resistant	Segregating	Susceptible	R:Seg:S[a] ratio	$\chi 2$ [b]	*P* value
Steenbras/Line37-07	68	78	8	7:8:1	0.293	0.863
Duzi/Line37-07	36	46	4	7:8:1	0.567	0.729
Tankwa/Line37-07	24	42	3	7:8:1	3.277	0.192
Krokodil/Line37-07	11	23	7	1:2:1	1.390	0.499
Betta/Line37-07	20	48	14	1:2:1	3.268	0.195

[a] R=resistant, Seg=segregating, S=susceptible

[b] Significant limit of $\chi 2$ ($p = 0.05$, $df = 2$)

Adult plant response ratios were determined at the peak of stem rust season (Line 37-07 > 60S)

Table 5.7: Molecular detection of *Sr* markers in selected South African wheat varieties.

Varieties	**csSr2** ***Sr2***	**Sr24#50** ***Sr24***	**Iag95** ***Sr31***	**Gwm319** ***Sr36***	**Gpw5182 Barc183** ***SrTmp***	**Cssfr5** ***Sr57***
Steenbras	1	0	0	0	0	0.5
SST 047	0	1	0	1	0	0
Duzi	0	1	0	0	0	1
Tankwa	0	0	1	0	0	0
Krokodil	0	0	0	0	0	0
Betta	0	1	0	0	0	0
Olifants	0	0	0	0	0	0
Line 37-07	0	0	0	0	0	0
CnsSrTmp	0	0	0	0	1	0
SST44	0	1	0	0	0	1
Agent	0	1	0	0	1	0
Betta R	0	0	0	0	1	0
Kingbird	1					
2S#2/163	0					
Kariega						1
Federation4*/Kavkaz			1			
Palmiet	1	1				
W2691SrTt-1 (W3528)				1		
CnsSrTmp					1	

Highlighted in grey are the seven control lines included for marker analysis

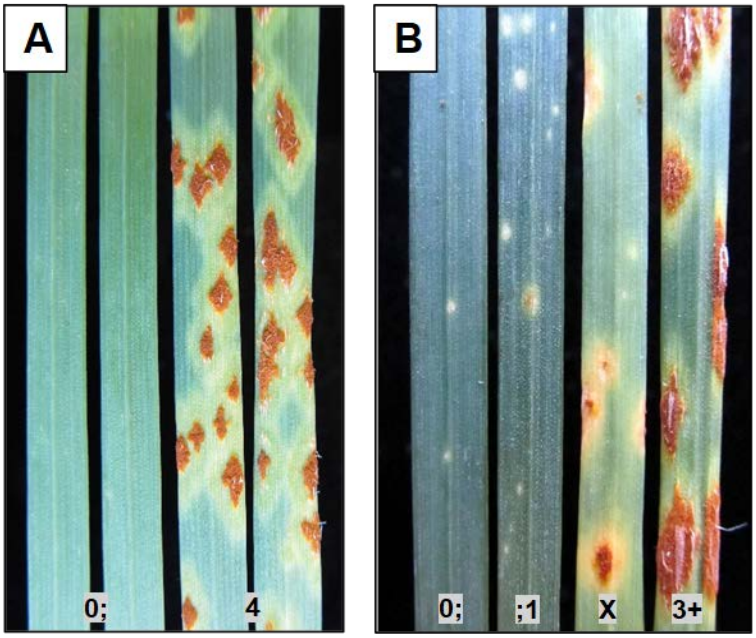

Figure 5.1: Seedling infection types (left to right, two leaves each) for parental lines Steenbras (0;) and Line 37-07(4) (A) and (B) segregation in the F2 population (0;, ;1, X, 3+) 14 days post inoculation with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

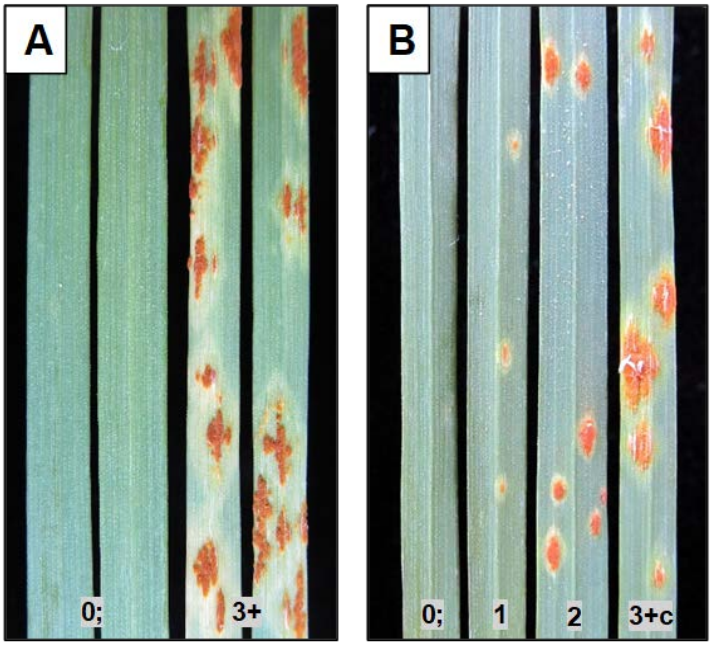

Figure 5.2: Seedling infection types (left to right, two leaves each) for parental lines SST 047 (0;) and Line 37-07(3+) (A) and (B) segregation in the F2 population (0;, 1, 2, 3+) 14 days post inoculation with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici*.

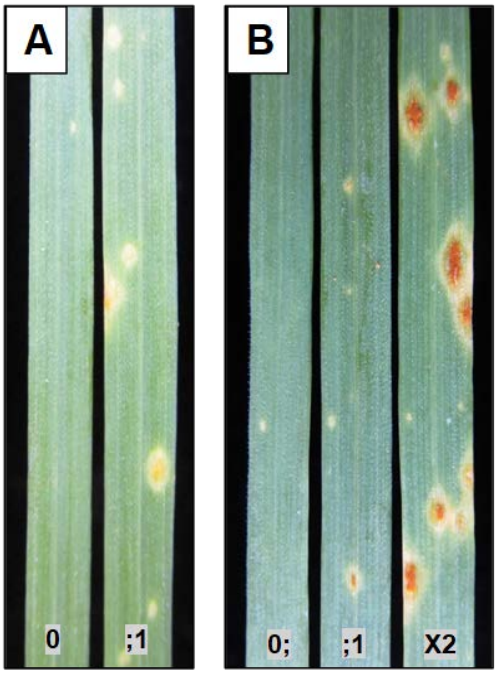

Figure 5.3: Seedling infection types for resistant F3 families from the cross Steenbras/Line37-07 (0;, ;1) (A) and (0;, ;1 and X2) (B) 14 days post inoculation with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici*.

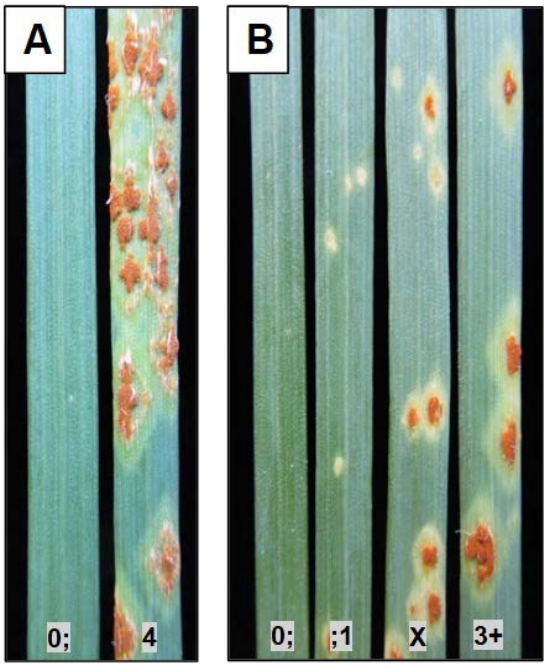

Figure 5.4: Seedling infection types for segregating F3 families from the cross Steenbras/Line37-07 (0;, 4) (A) and (0;, ;1, X, 3+) (B) 14 days post inoculation with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Figure 5.5: Seedling infection types for resistant F3 families from the cross SST047/Line37-07 (0;, ;1) (A) and (0;, ;1 and X) (B) 14 days post inoculation with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici*.

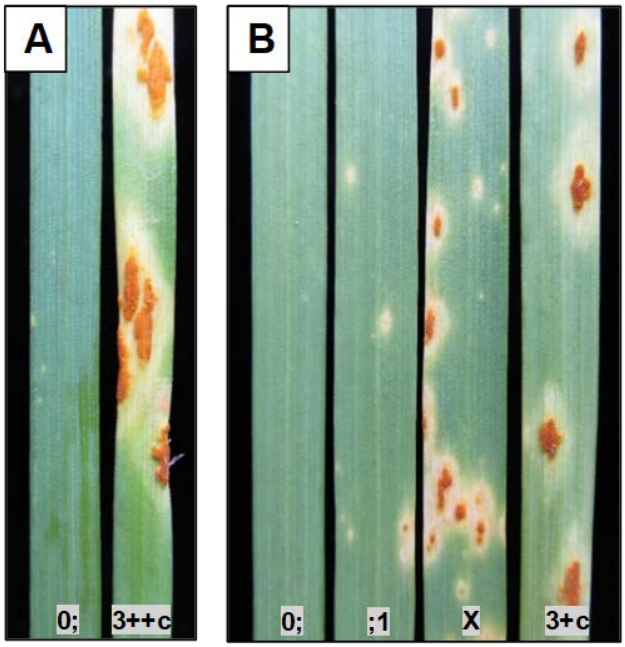

Figure 5.6: Seedling infection types for segregating F3 families from the cross SST047/Line37-07 (0;, 3++c) (A) and (0;, ;1, X, 3+c) (B) 14 days post inoculation with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

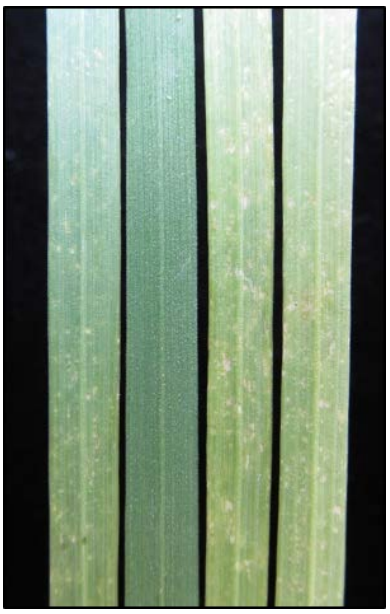

Figure 5.7: Seedling chlorosis (left to right, 1st leaf) on the primary leaf of Steenbras, Line 37-07 (2nd leaf, no chlorosis) and chlorosis on leaves 3 and 4 from two segregating F3 families for the cross Steenbras/Line37-07 14 days post inoculation with pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Figure 5.8: Expression of melanism, associated with the presence of *Sr2*, under the stem nodes of adult plants of the wheat cultivar Steenbras. Necrotic and chlorotic flecking visible above the stem nodes originated from a resistance response to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

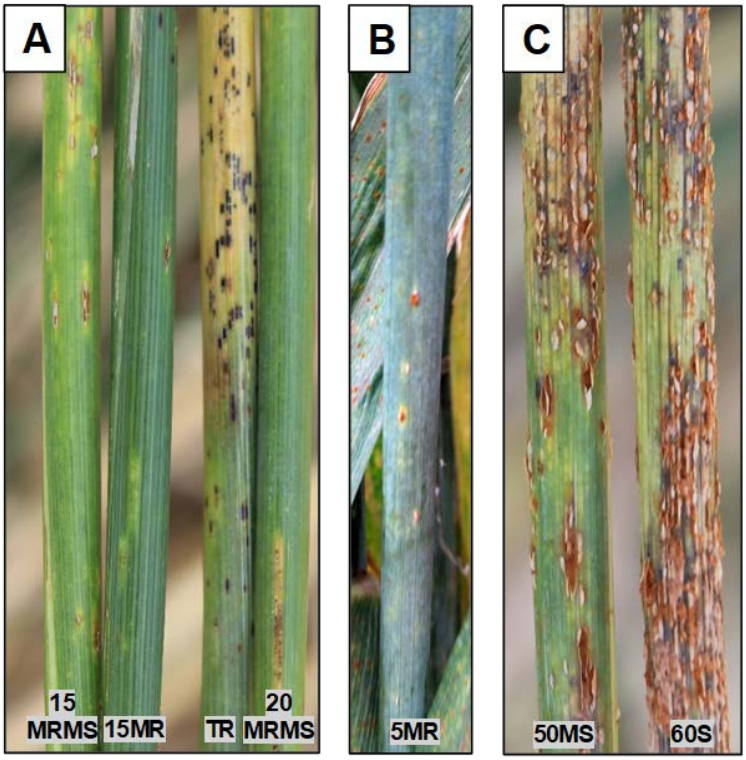

Figure 5.9: Adult plant responses to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici* on wheat stems in the field (from left to right) on resistant parental lines (A) Tankwa, Krokodil, Steenbras, Betta, (B) Duzi and susceptible parental lines (C) Olifants and Line 37-07, respectively.

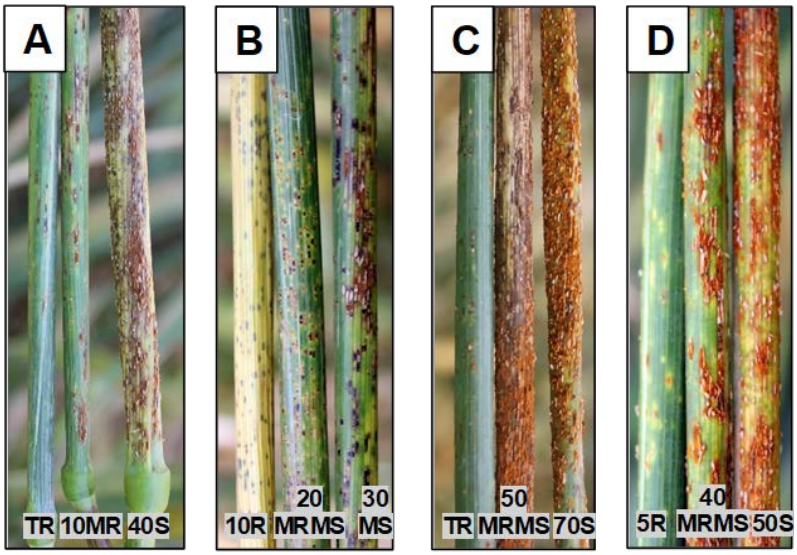

Figure 5.10: Adult plant responses to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici* on wheat stems in the field indicating resistant (left), intermediate (centre) and susceptible (right) reactions on each photo plate, respectively. Plates are representative of the F2 populations from the crosses Steenbras/Line37-07 (A), Steenbras/Olifants (B), Tankwa/Line37-07 (C), and Tankwa/Olifants (D).

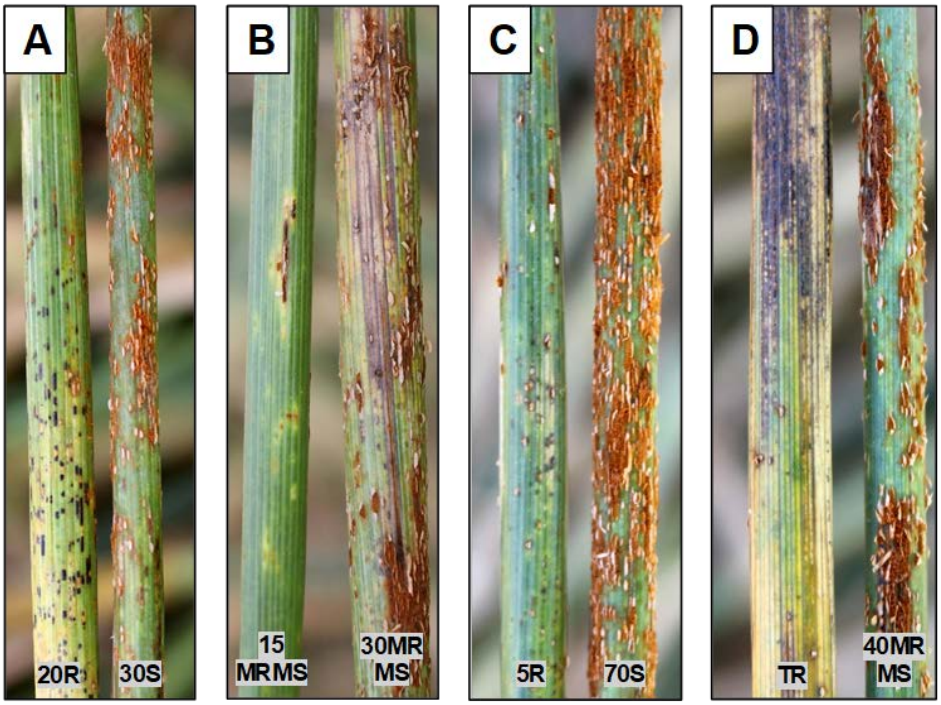

Figure 5.11: Adult plant responses to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici* on wheat stems in the field indicating resistant (left) and susceptible (right) reactions in the F2 populations from the crosses Krokodil/Line37-07 (A), Krokodil/Olifants (B), Betta/Line37-07, and (C) Betta/Olifants (D).

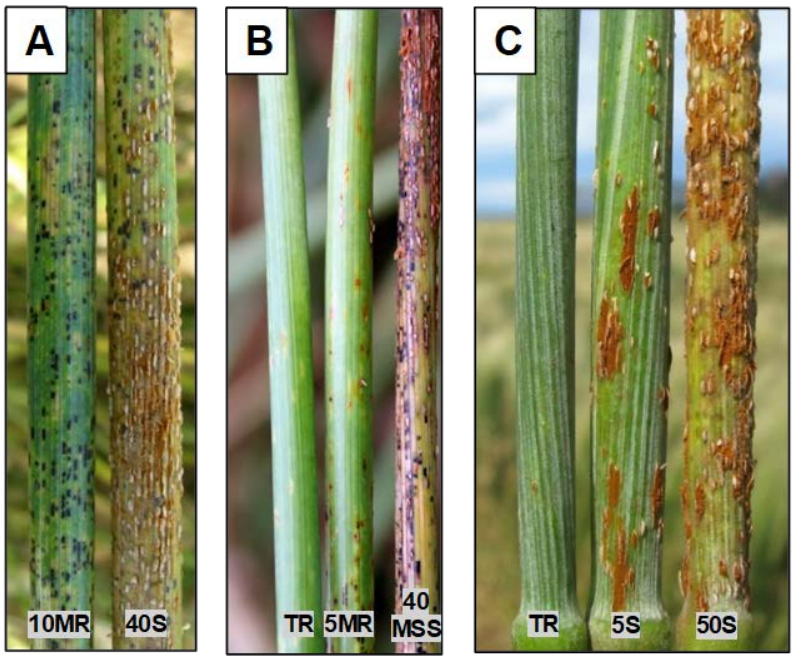

Figure 5.12: Adult plant responses to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici* on wheat stems in the field indicating resistant (left), intermediate (centre) and susceptible (right) reactions in the F3 families from the crosses Steenbras/Line37-07(A), Duzi/Line37-07 (B), and Tankwa/Line37-07 (C).

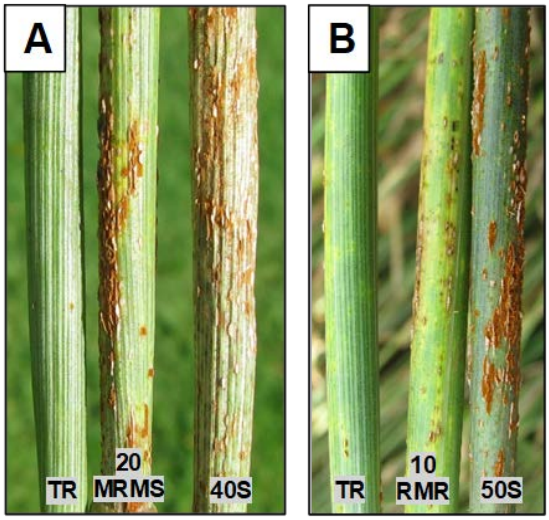

Figure 5.13: Adult plant responses to pathotype UVPgt60 of *Puccinia graminis* f. sp. *tritici* on wheat stems in the field indicating resistant (left), intermediate (centre) and susceptible reactions (right) in the F3 families from the crosses Krokodil/Line37-07(A) and Betta/Line37-07 (B).

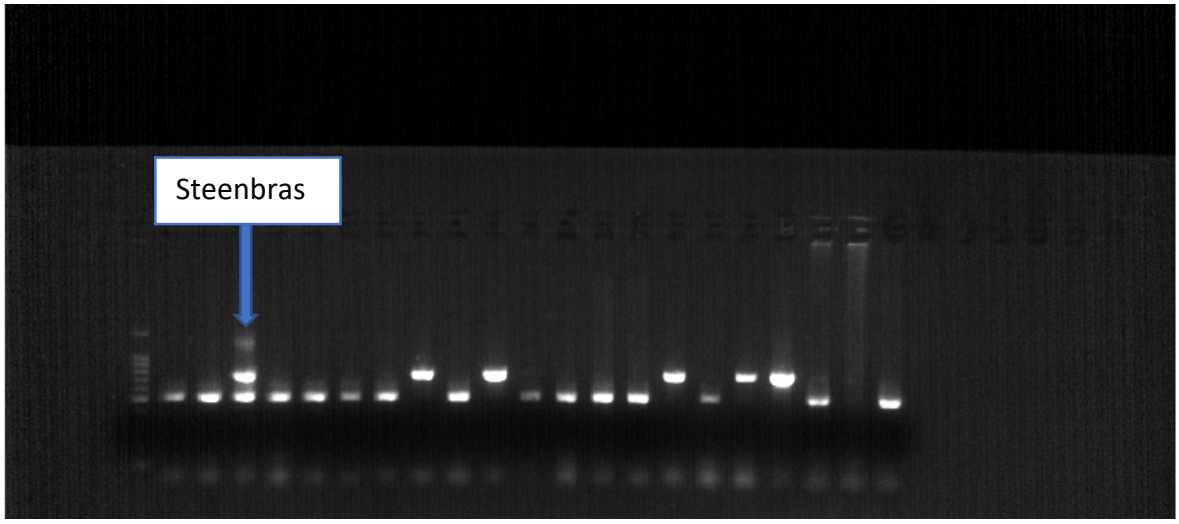

Figure 5.14: Agarose gel (1.5%) of marker cssfr5 linked to *Sr57*. Varieties from left to right 100bp ladder, Olifants, Line 37-07, Steenbras, Krokodil, Betta, Betta (repeat), Tankwa, Duzi, SST 047, SST 44, SrTmp, Agent, Betta R, Betta S, Kingbird, 2S#2/163, Kariega, Federation/Kavkaz, Palmiet, W2691SrTt-1 (W3528), Cns-SrTmp.

Chapter 6: An assessment of inheritance of stem rust resistance and infection in selected South African triticale cultivars

INTRODUCTION

Triticale (*xTriticosecale* Wittmack ex A. Camus; 2n = 6 × = 42), is a hybrid of wheat (*Triticum aestivum*) and rye (*Secale cereale*). Primary triticales result from intercrossing wheat and rye and secondary triticales are the product of crosses between primary types. Triticale was initially bred in Scotland and Sweden during the late 19th century to combine the yield potential and grain quality of wheat with disease and environmental tolerance of rye. Worldwide the crop is grown mostly for forage or fodder, although some triticale grain-based foods can be purchased from some health food stores. The protein content of triticale is higher than that of wheat but it has a lower glutenin fraction (Larter, 2012). Triticale breeding has mainly focused on the improvement of quantitative traits such as grain yield, nutritional quality and plant height. Unfortunately, little is known about disease resistance of rye. Initially biotic stresses were not a serious constraint to triticale production, but as the area planted increased, most wheat and rye diseases have started to affect production (Singh and Saari, 1991).

Wheat stem rust caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn (*Pgt*), emerged as a problem on triticale soon after commercial cultivation started (Singh and Saari, 1991). Resistance in triticale to stem rust has been reported (Adhikari and McIntosh, 1998, Singh and McIntosh, 1988, Zhang *et al*., 2010) and several resistance genes of rye origin such as *Sr27*, *SrNin*, *SrSatu, SrJ, SrBj* and *SrVen* have been described (McIntosh *et al.*, 1995). According to Adhikari and McIntosh (1998) two genes of wheat origin (*Sr9b* and *Sr36*) also play a role in some triticales. Virulence for *Sr27* has been reported in Australia (Singh and McIntosh, 1988), Madagascar (Singh and Saari, 1991) and South Africa (SA) (Smith and Le Roux, 1992). *Pgt* pathotypes documented on triticales in SA include UVPgt53 (BPGSC+*Sr27*, 1988), UVPgt56 (BPGSC+*Sr27*+*Kw*, 2003), UVPgt57 (BPGSC+*Sr27*+*Kw*+*Satu*, 2005), UVPgt58, (BNGSC+*Sr27*, 1988) and UVPgt62 (BFBSC+*Sr27*, 2010) (Boshoff *et al*., 2018, Terefe *et al*., 2016). Two other isolates

UVPgt54 (BNGSC, 2000) and UVPgt54+ (BPGSC, 2000) coded to race BNGSC, but were avirulent to *Sr27*, *SrKw* and *SrSatu* (Boshoff *et al*., 2018).

In SA triticale is grown mainly for animal feed but also as a cover crop in vineyards in the Western Cape (Fig 6.1) where conditions are usually favorable for stem rust development. The emergence of a new *Pgt* pathotype in 2005 rendered most of the South African triticales susceptible (Terefe *et al*., 2016). Race UVPgt57 is avirulent to *Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 30, 31, 36, 38, Tmp* and virulent for *Sr8a, 9a, 9b, 9d, 9g, 10, 11, 27, Kw, Satu, McN.* The vulnerability of South African triticale cultivars to new stem rust pathotypes therefore raised questions about the nature of resistance in cultivars such as Kiewiet and Tobie. In addition, the infection processes, early colonization and expression of stem rust resistance were investigated in triticale.

MATERIAL AND METHODS

Triticale and wheat genotypes

Cultivars included in this study were selected based on their resistance (Table 6.1) expressed to four South African *Pgt* pathotypes (Table 6.2). The SA triticale cultivars Tobie and Kiewiet were included together with the Australian cultivars Coorong (Inia-F-66/Armadillo) and Satu (Maya-2/Armadillo) and one USA entry Line *SrNin*. The wheat entry Line 37-07 selected from the 2nd International Stem Rust Trap Nursery (ISRTN07), entry 37, was used as a susceptible check based on its susceptibility to stem rust in local field trials. Tobie and Kiewiet were used as female parents and were crossed with Coorong (*Sr27*) and Satu (*SrSatu*) as male parents. Line *SrNin* was selected from entry 71 of the 7th International Stem Rust Trap Nursery (ISRTN07) to compare with Kiewiet. Line *SrNin* and Tobie were excluded from histological studies.

Parental seeds were planted in a 1:1 v/v sterilized soil/peatmoss mixture in 1L pots in the greenhouse, set to an 18-25°C night/day schedule. Seven days after emergence fertilization started by applying Multifeed® water-soluble fertilizer (19:8:16) NPK plus micronutrients) twice, followed by a water suspension of Wonder® 3:2:1, a slow nitrogen release granular fertilizer, at 7-day intervals until

seed set. Several crosses were made for each combination to ensure enough F1 seeds, but only ten F1 seeds from a single spike were selected, planted and to prevent out-crossing, spikes were covered before flowering with glassine crossing bags. F2 seeds were harvested at maturity, bulked and used for seedling phenotyping in the greenhouse.

Stem rust pathotypes

Seedlings were inoculated with freshly harvested urediniospores from *Pgt* pathotypes UVPgt53 (virulent to *Sr27* / avirulent to *SrSatu*, *SrKw* and Tobie), UVPgt56 (virulent to *Sr27* and *SrKw* / avirulent to *SrSatu* and Tobie), UVPgt57 (virulent to *Sr27*, *SrKw*, *SrSatu* and Tobie) and UVPgt60 (avirulent to *Sr27, SrKw*, *SrSatu* and Tobie). The first three races are avirulent to *Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 30, 31, 36, 38, Tmp* and virulent to *Sr8a, 9a, 9b, 9d, 9g, 10, 11, 27, McN,* while UVPgt60 (PTKST) is a member of the Ug99 race group and avirulent to the genes *Sr9h, 21, 22, 26, 33, 35, 36, 39,* and *Tmp* and virulent on *Sr5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 24, 30, 31, 38,* and *McN* (Terefe *et al*., 2016). Urediniospores were multiplied according to Knott (1989) on seedlings of the selective hosts (Table 6.2) to ensure race purity.

Seeds of the selective host for inoculum production were planted in 10-cm diameter plastic pots filled with Mikskaar® potting substrate MPS2. Emerging seedlings were drenched with 50 ml of a 99% maleic hydrazide ReagentPlus® [Sigma-Aldrich (0.3 g/L water) solution per 10 cm plastic pot to retard plant growth and enhance sporulation (Knott, 1989). Greenhouse temperatures were set to an 18-25°C night/day schedule and seedlings were fertilized one day before inoculation with 50 ml per pot of water-soluble Multifeed-Classic (Efekto®, NPK Analysis 19:8:16 (43), concentration 2.5 g/L water). After inoculation fertilization was applied twice a week until termination of trials.

Urediniospores (stored at -80°C) were heat shocked at 46°C for 6 min before spray-inoculation of seven-day-old seedlings of the respective multiplication hosts (approximately 3 mg spores per ml Soltrol® 130 (Chevron Phillips, Borger, Texas)). Leaves were spray-inoculated using a pressure pump at 25 kPa pressure setting

(Vacuubrand® pump - model MZ2) connected to an inoculation device. After inoculation seedlings were dried in a growth cabinet at 25°C for about 1 h before incubation in darkness in a dew simulation chamber at 18-23°C and >96% relative humidity for 16 h. From the dew chamber the seedlings were returned to a growth cabinet fitted with fluorescent growth tubes (Eurolux G135, G13 9W T8 LED) for at least 2 h. Seedlings were then transferred and maintained under previously described conditions in isolation cabinets in the greenhouse until sporulation and collection of spores for inoculation of either the F2 seedlings or adult plants.

Phenotyping

Seedlings - greenhouse

For F2 seedling screening, originating from the populations Tobie/Coorong, Tobie/Kiewiet, Tobie/Satu and Kiewiet/Coorong, Kiewiet/Satu and Satu/Coorong, approximately 200 seeds per population were planted, whereas for the Kiewiet/Line*SrNin* population 300 seedlings were screened. Fifteen seeds were planted per 10-cm plastic pot in Mikskaar® potting substrate MPS2. Greenhouse temperatures were set to an 18-25°C night/day schedule and plants fertilized with water soluble Multifeed-Classic (Efekto®, NPK Analysis 19:8:16 (43), concentration 2.5 g/L water) twice a week until termination of trials.

F2 populations were inoculated with freshly harvested urediniospores at the one and half leaf stage, approximately eight days after planting using an urediniospores suspension of ±1.5 mg/ml Soltrol® 130, Chevron Phillips, Borger, Texas. The same procedures as described, excluding maleic hydrazide, were performed for all seedling trials in evaluation of the F2 progenies. F2 seedlings and parental genotypes were inoculated with each of the stem rust races and maintained on a greenhouse bench until infection types (ITs) were recorded. Twelve days after inoculation seedlings were rated according to the 0-4 seedling infection type (SIT) scale, where 0 = immune, ; = flecks, c = chlorotic, or n = necrotic, 1 = minute uredinia, 2 = small to medium sized uredinia, 3 = large uredinia sometimes encircled by slim chlorosis, 4 = large uredinia without any chlorosis, X = random spreading of variable-sized uredinia on a single leaf, Y = ordered spreading of different sized uredinia with larger uredinia at leaf tip and Z = ordered spreading of different sized

uredinia with larger uredinia at leaf base (Roelfs *et al*., 1992). SITs of 3 or higher refer to susceptible (high IT) while 2 and lower and combinations thereof were considered resistant (a low IT).

Adult plants – greenhouse

For histological evaluations, five seeds (thinned out to three) were planted in 1 L plastic pots, filled with a sterilized peat/soil mixture, and for each variety, nine pots were planted. Planting was repeated 8 days later to synchronize plant growth stage at inoculation due to different growth periods of the varieties. After seedling emergence plants were fertilized twice a week with water soluble Multifeed-Classic (Efekto®, NPK Analysis 19:8:16 (43), concentration 2.5 g/L water), followed by a water suspension of Wonder® 3:2:1, a slow nitrogen release granular fertilizer, at 7-day intervals for the duration of the trial in the greenhouse with temperatures set to an 18-25°C night/day schedule. Plants were inoculated at the onset of flowering (Zadoks growth stage 61) (Zadoks *et al.*, 1974).

Freshly collected urediniospores were suspended in distilled water (1 mg/ml) and Tween 20® (0.03% v/v) and applied to stems according to the method developed by Bender *et al*., 2016, (Chapter 3) using a compressed air sprayer similar to the system described by Pretorius *et al*. (2007) for inoculating adult wheat plants with stripe rust. Plants were incubated for 24 h from 12:00 in a plastic dew chamber/tent in a greenhouse cubicle set at 18-25°C (night/day cycle). Upon removal, the plants were dried in the same cubicle and growth conditions were maintained as previously described. Stem rust responses were recorded 16 days after inoculation as severity ratings (Peterson *et al*., 1948) and infection types (Stubbs *et al*., 1986) (R=resistant, MR=moderately resistant, MS=moderately susceptible and S=susceptible including interpolations).

Histological analysis

The WGA-chitin method, named WAC assay by Ayliffe *et al*. (2013), was used for fungal biomass quantification of pathotypes UVPgt53, 56, 57 and UVPgt60 following inoculation of the three triticales and susceptible Line 37-07. *Pgt* growth and colony formation resulting from inoculation with pathotypes UVPgt53 and

UVPgt60 were viewed and quantified by fluorescence microscopy, whereas only plant material infected with pathotype UVPgt53 were sampled for scanning electron microscopy.

Scanning electron microscopy (SEM)

For each treatment, portions of the flag leaf sheath on the last stem internode were sampled, 24 and 48 hours post inoculation (hpi) and cut into segments (5 mm in length). Samples were fixed for at least 24 h in 3% (v/v) glutaraldehyde (in a 0.1 M sodium phosphate buffer at pH 7.0) according to the protocol described by Glauert (1974). Thereafter, samples were washed in 0.05 M phosphate buffer (2X) and post-fixed in 2% osmium tetroxide for 1 to 2 h, followed by rinsing twice with freshly prepared buffer for 20 min to dissolve the osmium tetroxide fixatives before it was dehydrated in a graded ethanol series (50%, 70% and 95% for 20 min in each phase followed by two changes of 100% for 1 h in each phase). The samples were critical-point-dried in a Tousimis critical point dryer (Maryland, USA), replacing the ethanol with pressurised liquid CO_2 at 31.5°C which ensure maximum structural preservation when the liquid CO_2 is converted to the gaseous phase.

The dried samples were directly mounted, using double sided carbon tape, on 12.2 mm diameter metal stubs (Cambridge pin type) for observations on the outer surface of the leaf sheath. Furthermore, segments were stripped according to a technique described by Hughes and Rijkengerg (1985) to view fungal development inside leaf tissues. The dried leaf sheaths were rolled open and mounted with the upper epidermis down on the stub, before the lower epidermis was removed by stripping.

The mounted leaf sheaths were sputter coated with gold (± 60 nm thickness) in a Bio-Rad sputter coater (United Kingdom). Specimens were examined with a JSM-7800F Extreme-resolution Analytical Field Emission SEM (Tokyo, Japan).

Fluorescence microscopy

Leaf stems obtained from the last stem internode of three plants per treatment were sampled 5 days after inoculation and cut into 1-cm sections. Stem segments were

processed according to a modified method of Ayliffe *et al*. (2011) using *Triticum vulgaris* lectin (wheat germ agglutinin) fluorescein isothiocyanate conjugate (WGA-FITC) (Sigma-Aldrich, St Louis, Missouri, USA). Stem segments were sampled in 1 M KOH and 0.05% (v/v) Silwet L-77 wetting agent (SouthernChem, Sandton, SA) and incubated at 37°C overnight (at least 16 h) to extract the chlorophyll. Thereafter, samples were washed twice with 50 mM Tris-HCl buffer (pH 7.0) and kept in buffer at 4°C until the stem segments turn yellow and semitransparent. After storage the samples were washed in freshly prepared 50 mM Tris-HCl buffer (pH 7.0) once again, before the leaf sheaths were carefully removed from the stems and stained for 16 h, at room temperature, with 8.3 µg/ml (w/v) WGA-FITC probe in the Tris-HCl buffer (pH 7.0). After staining, leaf sheaths were washed with Tris-HCl buffer (pH 7.0) and leaf sheath sections were used as whole mounts for fluorescence microscopy (Rohringer *et al*., 1977, Kuck *et al*., 1981).

Using the WU epifluorescence cube (450-480 nm excitation filter and 515 nm barrier filter) observations on 10 randomly selected infection sites on each of three leaf sheath segments were carried out at either X200 or X400 with an Olympus AX70 microscope (Tokyo, Japan) fitted with a CC12 digital camera for image capturing and measurements with Analysis LS Research version 2.2 software (Olympus Soft Imaging System, Japan) The area exhibiting fungal growth was measured in square micrometers (μm^2) and a mean calculated for each treatment. The number of haustorium mother cells (HMC) were counted in very small colonies (less than 30 HMC) or when necrosis was observed.

Fungal biomass assay

Rust infected tissue of the flag leaf sheaths, on the last stem internode for each of three plants per treatment, was harvested 16 days after inoculation and relative chitin biomas per mg fresh weight of the harvested tissue was determined by pooling leaf sheaths per plant and measuring the binding of wheat germ agglutinin-fluorescin isothiocyanate conjugate (WGA-FITC) (Sigma-Aldrich, St Louis, Missouri, USA).

Leaf sheaths were harvested, weighed and submerged in1 M KOH with a few µl of Silwet L-77 wetting agent (SouthernChem, Sandton, SA) added to keep the plant tissue submerged. The plant tissue were autoclaved for 15 min at 121°C under 15 psi of pressure and thereafter neutralized in 50 mM Tris (pH 7.0) by washing it twice for 15 min. Thereafter 50 mM Tris-HCl buffer (pH 7.0) was added with the final concentration of leaf tissue fixed at 200mg/ml. Tissue was sonicated for 60 seconds to attain a uniform and fine suspension and 10 µl WGA-FITC probe was added to 100 µl of sample and mixed by pipetting up and down. Sonicated tissue was incubated at room temperature for 30 min and then centrifuge at 1000 rpm for 3 minutes. The supernatant was removed and resuspended in 200 µl of 50 mM Tris-HCl buffer (pH 7.0). Three washes followed to remove unbound stain before transferring the sample to a 96 well optical bottom polymerbase black microtitre plate (Thermo Scientific Nunc, New York, USA) to quantify fluorescence for 1 s measurement time at 485 nm excitation and 535 nm emission in a Zenyth 3100 multimode detector (Anthos Labtec Instruments, Salzburg, Austria). Chitin measurements were repeated, for each treatment, and the average value is presented.

Data analysis

The Chi square test was applied to determine the goodness-of-fit to expected genetic ratios in the F2 generation. Analysis of data obtained in microscopy and the biomass assay was done with NCSS Statistical Software System (Hintze, 2007). A completely randomised design was used for data analysis.

RESULTS

Phenotyping

Seedlings – greenhouse

Disease reactions, rated according to a 0-4 rust SIT scale, showed that only Coorong was susceptible to UVPgt53 (Fig 6.2 A) among the triticale cultivars, whereas UVPgt56 (Fig 6.2 B) produced high SITs on Coorong and Kiewiet. All triticale cultivars were susceptible to UVPgt57 (Fig 6.2 C), but resistant to UVPgt60 (Fig 6.2 D). Line *SrNin* was resistant to all pathotypes tested (Fig 6.3) with SITs that

varied from 0; with pathotype UVPgt60 to a 1 with UVPgt57. Seedling ITs for Kiewiet were 3+ and 4 with pathotypes UVPgt56 and UVPgt57 respectively.

F2 seedlings from the Tobie/Coorong population segregated with typical SITs that varied from ;1, 2++ to 3+ for pathotype UVPgt53 (Fig 6.4 A) and from 0;, ;1, 2 to 4 with pathotype UVPgt56 (Fig 6.4 B). Segregation ratios observed in F2 seedlings confirmed a single resistance gene in the Tobie/Coorong cross for both UVPgt53 ($\chi_{3:1}$ = 0.886, p = 0.346) and UVPgt56 ($\chi_{3:1}$ = 1.918, p = 0.166). The Tobie/Kiewiet cross segregated for two resistance genes with UVPgt53 ($\chi_{15:1}$ = 3.135, p = 0.077) and for one gene with UVPgt56 ($\chi_{3:1}$ = 0.443, p = 0.505) (Table 6.3 and 6.4). The SITs for offspring from the Tobie/Kiewiet cross ranged from 0;, ;1, 1+c to 3 with pathotype UVPgt53 (Fig 6.5 A) and for UVPgt56 from ;1=c to 4 (Fig 6.5 B). No susceptible plants were observed in Tobie/Satu offspring inoculated with either UVPgt53 or UVPgt56, indicating that these varieties have a gene in common (Table 6.3 and 6.4). Evaluations of F2 seedlings with pathotype UVPgt53 from the cross Kiewiet/Coorong ($\chi_{3:1}$ = 0.843, p = 0.358), where SIT ranged from 1c, 2 to 3+ (Fig 6.6 A), indicated the presence of one *Sr* gene. However, with pathotype UVPgt56 intermediate SITs of 2++ observed for 30 seedlings (Fig 6.6 B) in addition to a majority of clearly susceptible seedlings (159, SIT 4) were unexpected and did not conform to a particular resistance gene ratio fit (Table 6.4). The offspring from the cross Kiewiet/Satu ($\chi_{15:1}$ = 0.082, p = 0.775) segregated for two stem rust resistance genes with SITs varying from ;1-c, 2- to 3++ with pathotype UVPgt53 (Fig 6.7 A), whereas with pathotype UVPgt56 (Fig 6.7 B) SITs varied from 0;, ;1 to 4 and only one resistance gene is postulated ($\chi_{3:1}$ = 0.674, p = 0.412). A single resistance gene, effective to UVPgt53 ($\chi_{3:1}$ = 0.496, p = 0.481) and UVPgt56 ($\chi_{3:1}$ = 0.156, p = 0.693) was identified in the Satu/Coorong cross (Table 6.3 and 6.4). SITs ranging from ;1= to 3++ and from ;1= to 4 were recorded with pathotypes UVPgt53 and UVPgt56 respectively (Fig 6.8). As expected all F2 populations inoculated with UVPgt57 yielded only susceptible seedlings.

The evaluation of F2 seedlings from the Kiewiet/Line*SrNin* cross (Table 6.5) yielded unexpected results and therefore an extended population was tested. With both pathotypes UVPgt53 (SIT ;;1) and UVPgt60 (SIT ;) only resistant seedlings were

observed (Fig 6.9 A and B) whereas the offspring segregated for two stem rust resistance genes when inoculated with both pathotypes UVPgt56 ($\chi_{15:1}$ = 0.36036, p = 0.54830) and UVPgt57 ($\chi_{15:1}$ = 0.12973, p = 0.71871). Although SITs only varied between 22+ and 3+ for pathotype UVPgt56 (Fig 6.10 A) and from 22+ to X3 and 3+ (Fig 6.10 B) the distinction was clearly notable between resistance and susceptibility.

Adult plants – greenhouse

Sixteen days after inoculation, adult plant response types observed on the stems of the triticales inoculated with pathotype UVPgt53 (Fig 6.11 A) varied from 0R for Satu to 30R for Kiewiet with a susceptible response type of 70S recorded for Coorong. Only Satu was resistant (0R) to pathotype UVPgt56 (Fig 6.11 B), whereas with UVPgt60 all the triticales showed low response types (Fig 6.11 C). Line 37-07, used as susceptible control, ranged as expected from 40MSS (UVPgt56), 60MSS (UVPgt53) to 30S (UVPgt60).

Histological analysis

Scanning electron microscopy (SEM)

The early infection process of *Pgt* examined on leaf sheaths by SEM allowed a comparison between triticale and wheat. At germination of the urediniospore the germ tube protrudes from the germ pore, sometimes from both longitudinal sides of the urediniospore (Fig 6.12 A) and extends, normally perpendicularly to the stem long axis (Fig 6.12 B) until a stoma is encountered and an appressorium is formed above the stomatal opening (Fig 6.12 C). As the appresorium matures it is delimited from the germ tube by a septum (Fig 6.12 D) and the germtube collapse. At 48 hpi most of the appressoria had collapsed on top of the stomata (Fig 6.13 A). No significant differences were observed between the appearances of the appressoria on susceptible wheat or triticales compared to that on resistant triticales.

The blade-like connection (Fig 6.13 B), previously described as the interconnective tube (Hughes and Rijkenberg, 1985, Lennox and Rijkenberg, 1989) links the appresorium with the susbtomatal vesicle (SSV) that develops inside the substomatal chamber. The SSV initially appears as an oblong structure from the

stomatal slit (Fig 6.14 A, B and C), elongates to the sides either into a primary infection hyphae (PIH) (Fig 6.14 C) or a haustorium mother cell (HMC) without the formation of a PIH (Fig 6.15 A). A septum is formed to delimit the HMC (Fig 6.15 B). When the mature HMC comes into contact with the host mesophyll cell an infection peg (Fig 6.15 C) is formed that, via enzymatic actions, penetrates the mesophyll cell to form haustoria for nutrient extraction. No obvious differences were observed in early infection structure development such as SSV in Line 37-07 as opposed to resistant Satu or Kiewiet (Fig 6.14 A, B and C) at 24 hpi.

HMCs were frequently observed at 48 hpi (Fig 6.16 A, B and C) together with the first secondary infection hypha that usually arises in close proximity to the HMC. Generally two secondary infection hyphae were observed emerging together in Coorong (Fig 6.16 B). The absence of a HMC could possibly be associated with an observed collapsed SSV at 48 hpi in the resistant triticale cultivar Satu (Fig 6.16 D).

Fluorescence microscopy

Pgt detection was enabled by the binding of the fungal chitin with the WGA-FITC probe with little background fluorescence. Infection structures of *Pgt* observed at 120 hpi showed established colonies of pathotype UVPgt53 in Line 37-07 and Coorong (Fig 6.17 A and B). In Kiewiet small colonies (Fig 6.17 C), 12.5 ± 3 HMC, were observed, but more than 81.9% of infection sites indicated only one or two HMC. In Satu no HMC were visible only the SSV (Fig 6.17 D). Some infection sites of *Pgt* in Kiewiet were visibly associated with host cell necrosis (HCN) (Fig 6.18), but staining was not developed for HCN evaluation and therefore did not allow a quantitative assessment of necrosis. Pathotype UVPgt60 of *Pgt* established colonies only in Line 37-07 (Fig 6.19 A), whereas in Coorong only appressoria with no HMC were visible (Fig 6.19 B). In both Kiewiet and Satu a HMC was occasionally observed (Fig 6.20 A, B, C and D), but HCN was only evident in Kiewiet (Fig 6.20 B). Colony size (μm^2) was significantly ($P<0.05$) influenced by host genotype and pathotype, but did not differ significantly ($P<0.05$) between the three harvested sheaths representing replicates per treatment, therefore only the means are demonstrated in Figure 6.21.

Fungal biomass assay

Successful binding of WGA-FITC to chitin in fungal tissue resulted in excessive measurement of fluorescence in susceptible lines with the maximum amount of 1049954 in Line 37-07 inoculated with pathotype UVPgt53, whereas in resistant lines reduced fluorescence measurements ranged from 76590 to 95843, indicating less chitin. Due to uniform inoculation of wheat stems measurements of chitin in plant leaf tissue did not differ significantly ($P<0.05$) (Table 6.6) between the two trial replications or the three harvested sheaths per trial, therefore only the means are demonstrated in Figure 6.22. Significant variation ($P<0.05$) occurred among entries and pathotypes as well as the interaction thereof. Although Kiewiet was resistant (30R) an increased amount of fluorescence (660022.5) with pathotype UVPgt53 was observed.

DISCUSSION

Inheritance

Although previous results by Olivera *et al*. (2013) established triticale as an ample resistance source to TTKSK, the original Ug99 race, other stem rust resistance studies (McIntosh *et al*., 1983, McIntosh, 1988, Singh and McIntosh, 1988, Zhang *et al*., 2010) documented a very narrow genetic base in the crop. The failure of stem rust resistance in Kiewiet and Tobie questioned the genetic base of resistance in these two South African triticale cultivars and underlined the fact that broad-based rust resistance was not a priority during cultivar release. Given the complexity of traits required by the various industries for the release of small grain cultivars, the unintentional occurrence of monogenic rust resistance is not unusual. In regions where stem rust is endemic, breeders should however be aware that new cultivars could be vulnerable to pathotype changes, leading to the well-known boom-and-bust scenarios.

A high percentage of international triticale accessions (141 from 353) tested by Olivera *et al*. (2013) were postulated to carry the resistance genes *Sr27*, *SrKw* and *SrSatu*. CIMMYT materials played a major role worldwide in the improvement and breeding of triticale (Zhang *et al*., 2010). Coorong and Satu respectively carry *Sr27* and *SrSatu* and represent the two most common stem rust resistance genes

present in CIMMYT lines (McIntosh, 1988, McIntosh *et al*., 1983, Singh and McIntosh, 1988, Zwer *et al*., 1992). McIntosh *et al*. (1983) indicated that more than 70% of the entries in 12th International Triticale Screening Nursery from CIMMYT, Mexico carried *Sr27*. However, virulence to *Sr27* was already present in SA in 1988 (Smith and Le Roux, 1992) and, although it had almost no influence on wheat production, it had a major impact on commercial triticale production. Furthermore, virulence was detected in SA for both *SrKw* in 2003 and for *SrSatu* in 2005 (Terefe *et al*., 2016). According to Terefe *et al*. (2010) one of the reasons for the common occurrence of these closely related *Pgt* pathotypes, UVPgt53, 56 and UVPgt57, in the Western Cape is due to the presence of corresponding single rust resistance genes in South African triticale. Growing susceptible triticale as pasture or cover crops during the dry summer months in the Western Cape, where environmental conditions favour stem rust development, undoubtedly contributes to the survival of these pathotypes that might lead to severe infection.

The stem rust resistance genes *SrSatu* and *Sr27* are described as all stage resistance genes (McIntosh, 1988, Adhikari, 1996, Zhang *et al*., 2010). Singh and McIntosh (1990) confirmed *SrSatu* in approximately 50% of entries included in the 17th International Triticale Screening Nursery. Pathotype UVPgt57 (virulent for *Sr27*, *SrKw* and *SrSatu*) was first identified on Tobie, a South African bred spring triticale cultivar, in the Western Cape during 2005 (Roux *et al*., 2006). In the present study a single gene was confirmed to occur in both Tobie and Satu and since no susceptible plants were observed in F2 offspring of the cross Tobie/Satu inoculated with either UVPgt53 (virulence to *Sr27*) or UVPgt56 (virulence to *Sr27* and *SrKw*), it is concluded that these varieties have the *SrSatu* gene in common.

Kiewiet carries an uncharacterized gene named *SrKw* (Olivera *et al*., 2013) and segregation analysis in this study confirmed that the resistance in Kiewiet is controlled by a single dominant gene. Kiewiet (*SrKw*) x Coorong (*Sr27*) offspring segregated only for *SrKw* when inoculated with pathotype UVPgt53 (virulence to *Sr27*), but when crossed with either Tobie or Satu two major stem rust resistance genes (*SrKw* and *SrSatu*) were expressed. However, when the F2 population from the Kiewiet/Coorong cross was tested with pathotype UVPgt56 (virulence to *Sr27*

and *SrKw*) only susceptible offspring was expected considering the reaction of the two parents to this race. The presence of seedlings with an intermediate SIT is difficult to clarify and may indicate the presence of a second, previously unnoted moderate-effect resistance gene which segregated in the population, or some form of residual resistance resulting from this particular parent combination.

The stem rust resistance gene *SrNin* from rye present in ‘Ningadhu” occurs at relative high frequencies in CIMMYT germplasm (Adhikari and McIntosh, 1998). Together with *SrBj* present in Bejon, only these two genes provided effective resistance in Australia (Adhikari and McIntosh, 1998). Kankwatsa *et al.* (2018) demonstrated that most Australian pathotypes are avirulent to *SrNin* (SITs ; to ;1-) and in Iran similar results were obtained for *SrNin* (SITs 0 to ;1=) (Nazari and Mafi, 2013). Both *SrNin* and *SrBj* are located in chromosome 2R that are valued important as stem rust resistance gene bearers in triticale (Tyrka and Chelkowski, 2004). A viable hypothesis in formal genetic studies of resistance genes located in rye chromosome 2R by Adhikari and McIntosh (1998) was “irregular inheritance patterns might reflect the behavior of an unpaired rye chromosome in competition with and unpaired 2D”. *SrNin* is usually combined in triticale with *SrSatu* and less frequently with *Sr27* (McIntosh *et al*., 1995, Adhikari and McIntosh, 1998). However, both these genes and *SrKw* are not effective against the pathotype UVPgt57 and despite this two resistance genes were expressed in Line *SrNin* against UVPgt56 and UVPgt57. The similarity of the resistant response type of pathotype UVPgt60 (member if the Ug99 race group) on *SrNin* and Kiewiet raised the hypothesis that these lines have a gene in common. However, despite the similar phenotype multipathotype studies indicated that the Kiewiet gene is most likely not involved and the resistance in Line *SrNin* to UVPgt56 and UVPgt57 is based on *SrNin* plus an additional seedling resistance gene. Pedigrees of triticale accessions typically are complicated or unknown. Therefore, it is almost impossible to postulate gene-for-gene relationships between rust pathotype and triticale host.

The analysis of stem rust resistance in the South African triticale cultivars confirmed simply inherited, race-specific resistance in both Kiewiet and Tobie, explaining the short-lived nature of resistance to *Pgt* in these varieties. The identity of the Kiewiet

gene is not known as opposed to confirmation of *SrSatu* in Tobie.

Infection process

According to Leonard and Szabo (2005) *P. graminis* has a very broad host range of over 300 different grass species, including wheat and rye, the two parents of triticale. To add to our understanding of the host-pathogen interaction, which is the basis of disease phenotype observed as signs and symptoms, histological investigations elucidate the infection structures and subsequent colonisation process of rust pathogens in their cereal hosts. According to available literature, no comprehensive information exists for the infection process of *Pgt* on triticale and whether infection structure differentiation in this pathosystem differs from other small grain hosts.

The establishment of *Pgt* firstly depends on spore germination and thereafter the location of a stoma by the germtube. Previous rust studies on barley, cowpeas and wheat indicated no differences in spore germination between resistant and susceptible host plants (Heath, 1974, Harder *et al*., 1978, Jacobs, 1989a, Niks, 1982, 1983). Appressorium formation is a prerequisite for the infection process and the precision of directional growth towards the stoma has been recognized since 1928 (Allen, 1928). Pre-penetration stages in the development of wheat stem rust are germination, germtube growth and formation of an appressorium over a stoma (Littlefield and Heath, 1979, Jacobs, 1989a). Using SEM no obvious morphological differences in pre-penetration fungal behavior were observed between susceptible wheat and either susceptible or resistant triticale lines. This indicated that the epidermal morphology of triticale stems did not influence the early infection structures of *Pgt* any differently from growth and differentiation on its wheat host.

The visible blade like structure between the appressorium and the SSV was named the interconnective tube (Lennox and Rijkenberg, 1989, Hu and Rijkenberg, 1998). Inside the stomatal cavity, a substomatal vesicle emerges from the stomatal slit usually as a long-spherical structure (Castelyn, 2018, Hu and Rijkenberg, 1998, Maree, 2018). Similar to previous studies the SSV elongated either into a HMC that is separated by a septum or a primary infection hyphae (Castelyn, 2018, Maree,

2018). As previously hypothesized (Heath and Skalamera, 1997), this study demonstrates the formation of an infection peg that is initiated when a hyphal tip contacts a host cell. After penetration the haustorium develops distally from the narrow neck inside the mesophyll cell to extract nutrients from the host (Voegele *et al*., 2009). Secondary infection hyphae, developing directly from the SSV, were rarely observed at 48 hpi due to slow rust development.

At 48 hpi most appressoria appeared collapsed on the stoma of both wheat and triticale, however, according to Chakravorty and Scott (1982), gene expression is triggered at a very early stage of infection and at 48 hpi, mostly collapsed substomatal vesicles were visible in the resistant Satu line. Although the rapid triggering of a hypersensitive reaction by rust spores (Kleinhofs *et al*., 2009) is at times associated with resistant lines, no necrotic cells were observed at infection sites of Satu. According to Heath (1982), most infection hyphae in resistant or incompatible hosts produce at least one haustorium mother cell, irrespective of the infection type that eventually develops.

Overall, infection structure formation on and in susceptible wheat, and susceptible and resistant triticale lines, is similar to that observed in previous studies with barley, sorghum, maize and wheat (Hu and Rijkenberg, 1998, Lennox and Rijkenberg, 1989, Castelyn, 2018, Maree, 2018), although slower rust development was observed in this study in sheaths of all varieties.

Establishing resistance or susceptibility usually includes interactions that involve different degrees of *Pgt* growth and host response, measurable as pustule size and biomass of fungal growth. The adult plant response of 0R in Satu conforms to the fluorescence microscopy observations where only SSV and no HMC formation were observed 5 dpi. Colony size was significantly influenced by host genotype and pathotype as evidenced by the susceptible Line 37-07 hosting large colonies of both pathotypes, but Coorong only with pathotype UVPgt53. Host cell necrosis was occasionally visible in Kiewiet inoculated with pathotype UVpgt53 that corresponded with the adult plant response of 30R and chlorosis visible on leaf sheaths. The onset of necrosis was noticed immediately after contact of HMC with

the host, however small colonies developed at other infection points in Kiewiet. According to Jacobs (1989b) cells often collapsed after penetration of the cell wall and the formation of a haustorium, and these observations suggested that posthaustorial collapse of cells may contribute to the abortion of infection structures, therefore fewer and smaller colonies.

The WAC assay method (Ayliffe *et al*., 2013) was successfully used to analyze *Pgt* biomass in the leaf sheaths of inoculated adult plants, when the WGA-FITC bound with chitin resulted in clear differences between resistant and susceptible lines with all pathotypes. Low variation between replications and sheaths supports the use of the WAC assay for chitin quantification in adult plants of triticale inoculated with *Pgt*. Previous studies on wheat by Castelyn (2018) demonstrated that the onset of APR can effectively restrict *Pgt* colonization in sheaths, however the results from this study revealed that an APR response of 30R in Kiewiet with pathotype UVPgt53 yielded a relative high amount of fungal growth. Distinguishing susceptible wheat lines by colony size at 5 dpi was comparable to chitin quantification (at a later stage) in the same trials. Both methods indicated Line 37-07 as most susceptible to the *Pgt* pathotypes included and Satu as resistant. Although the results for Kiewiet indicated relatively smaller and fewer colonies, it resulted in an intermediate amount of fungal growth.

In conclusion, this study confirmed the vulnerable stem rust resistance base in local triticale cultivars, emphasising the need for improved selection procedures. Furthermore, methods commonly used for studying histopathology of stem rust in wheat are equally applicable to triticale.

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Zwer, P.K., Park, R.F. and R.A. McIntosh. 1992. Wheat stem rust in Australia 1969-1985. Australian Journal of Agricultural Research 43, 399-431.

Table 6.1: Origin, status and seedling infection types of the parental genotypes to pathotypes UVPgt53, 56, 57 and UVPgt60 of *Puccinia graminis* f. sp. *tritici.*

Parents of the crosses	Status	Origin	UVPgt53[a]	UVPgt56[a]	UVPgt57[a]	UVPgt60[a]	Postulated known *R* gene
Tobie	Cultivar	South Africa	;;c	;;c	4	0;	
Kiewiet	Cultivar	South Africa	1^{+c}	3^{+}	4	$0;^{c}$	*SrKw*
Satu	Cultivar	Australia	0;	0;	4	0;	*SrSatu*
Coorong	Cultivar	Australia	4	4	4	0;	*Sr27*
SrNin	Line	ISRTN07 USA	;;1	;;1	1	0;	*SrNin*
Line 37-07*	Line	ISRTN07 USA	4	4	4	4	

[a] Seedling infection types were determined 12 days after inoculation

* Used as susceptible control

Table 6.2: Avirulence/virulence formulas for *Puccinia graminis* f. sp. *tritici* pathotypes used to determine host response of South African triticales.

NA code[a]	UFS code[a]	SA code[a]	Avirulence/Virulence *Sr* genes[b*]	Selective hosts[c]
BPGSC+*Sr27*	UVPgt53	2SA102	*Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 30, 31, 36, 38, Kw, Satu, Tmp*/*8a, 9a, 9b, 9d, 9g, 10, 11 ,27, McN*	Coorong
BPGSC+*Sr27*+*Kw*	UVPgt56	2SA104	*Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 30, 31, 36, 38, Satu, Tmp*/*8a, 9a, 9b, 9d, 9g, 10, 11, 27, Kw, McN*	Kiewiet
BPGSC+*Sr27*+*Kw*+*Satu*	UVPgt57	2SA105	*Sr5, 6, 7b, 8b, 9e, 17, 21, 24, 30, 31, 36, 38, Tmp*/*8a, 9a, 9b, 9d, 9g, 10, 11, 27, Kw, Satu, McN*	Kiewiet or Satu
PTKST	UVPgt60	2SA107	*Sr9h, 21, 27, 36, Kw, Satu, Tmp*/*5, 6, 7b, 8a, 8b, 9a, 9b, 9d, 9e, 9g, 10, 11, 17, 24, 30, 31, 38, McN*	Fed4*/Kavkaz

[a]Pathotypes were named according to North American (NA) nomenclature (Roelfs and Martens, 1988). University of the Free State (UFS) and South African (SA) codes

[b]Avirulence/virulence according to Terefe *et al*., 2016 and Boshoff *et al*., 2018.

[c]Selective hosts on which pathotypes were increased. Kiewiet (*SrKw*), Satu (*SrSatu*), Coorong (*Sr27*) and Fed4*/Kavkaz (*Sr31*)

Table 6.3: Segregation ratios of F2 populations determined in the greenhouse on seedlings inoculated with pathotype UVPgt53 of *Puccinia graminis* f. sp. *tritici.*

Cross	Resistant	Intermediate	Susceptible	R:S[a] ratio	χ^2	*p*
Tobie/Coorong	142	14	26	3:1	0.886	0.346
Tobie/Kiewiet	142	21	17	15:1	3.135	0.0766
Tobie/Satu	160					
Kiewiet/Coorong		176	51	3:1	0.843	0.358
Kiewiet/Satu	181	21	12	15:1	0.082	0.775
Satu/Coorong	133		39	3:1	0.496	0.481

[a] R=resistant and intermediate, S=susceptible
Seedling infection types were determined 12 days after inoculation

Table 6.4: Segregation ratios of F2 populations determined in the greenhouse on seedlings inoculated with pathotype UVPgt56 of *Puccinia graminis* f. sp. *tritici.*

Cross	Resistant	Segregating	Susceptible	R:Seg:S[a] ratio	χ^2	*p*
Tobie/Coorong	133	17	17	3:1	1.918	0.166
Tobie/Kiewiet	92		35	3:1	0.443	0.505
Tobie/Satu	180					
Kiewiet/Coorong	30		159			
Kiewiet/Satu	103		40	3:1	0.674	0.412
Satu/Coorong	132		41	3:1	0.156	0.693

[a] R=resistant, Seg=segregating, S=susceptible
Seedling infection types were determined 12 days after inoculation

Table 6.5: Segregation ratios of F2 seedlings for the cross Kiewiet/Line*SrNin* determined in the greenhouse with four pathotypes of *Puccinia graminis* f. sp. *tritici.*

Cross	Resistant	Intermediate	Susceptible	R:S[a] ratio	χ^2	*p*
UVPgt53	328					
UVPgt56		275	21	15:1	0.36036	0.54830
UVPgt57		276	20	15:1	0.12973	0.71871
UVPgt60	305					

[a] R=resistant and intermediate, S=susceptible

Seedling infection types were determined 12 days after inoculation

Table 6.6: Analysis of variance for measurements of the WGA-FITC probe (wheat germ agglutinin fluorescein isothiocyanate conjugate) bound with fungal chitin for pathotypes UVPgt53, 56 and UVPgt60 of *Puccinia graminis* f. sp. *tritici* measured 16 dpi for the susceptible control Line 37-07 and triticales Coorong, Kiewiet and Satu.

Source Term	df	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Race	2	1.174402E+12	5.872009E+11	54.21	0.000000*	1.000000
B: Line	3	2.657865E+12	8.859551E+11	81.79	0.000000*	1.000000
AB	6	2.045203E+12	3.408672E+11	31.47	0.000000*	1.000000
S	24	2.599822E+11	1.083259E+10			
Total (Adjusted)	35	6.137452E+12				
Total	36					

* Term significant at alpha = 0.05

Least significant difference between lines = 101267.38

Figure 6.1: Triticale grown as cover crop in vineyards in the Western Cape, South Africa.

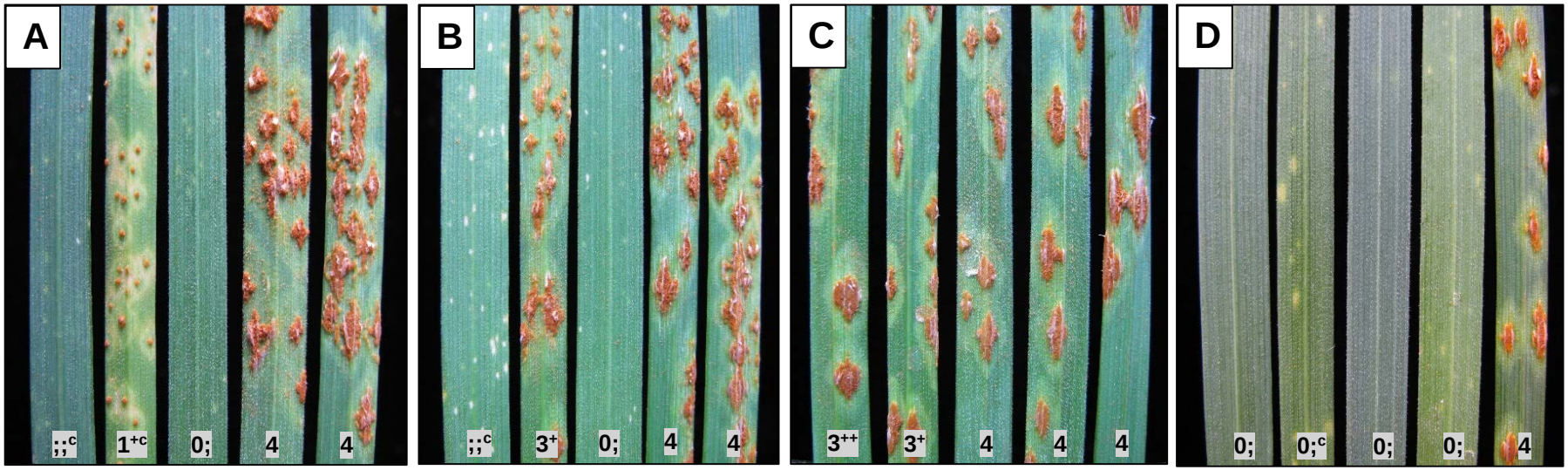

Figure 6.2: Seedling infection types for parental lines Tobie, Kiewiet, Satu, Coorong and Line 37-07 (left to right) 12 days post inoculation with pathotypes (A) UVPgt53, (B) UVPgt56, (C) UVPgt57 and (D) UVPgt60 of *Puccinia graminis* f. sp. *tritici*.

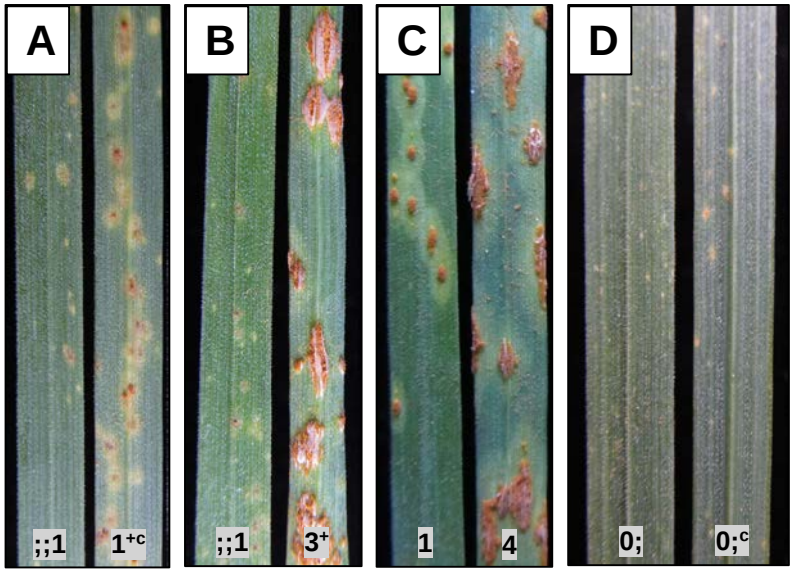

Figure 6.3: Seedling infection types for parental lines SrNin and Kiewiet (left to right) 12 days post inoculation with pathotypes (A) UVPgt53, (B) UVPgt56, (C) UVPgt57 and (D) UVPgt60 of *Puccinia graminis* f. sp. *tritici*.

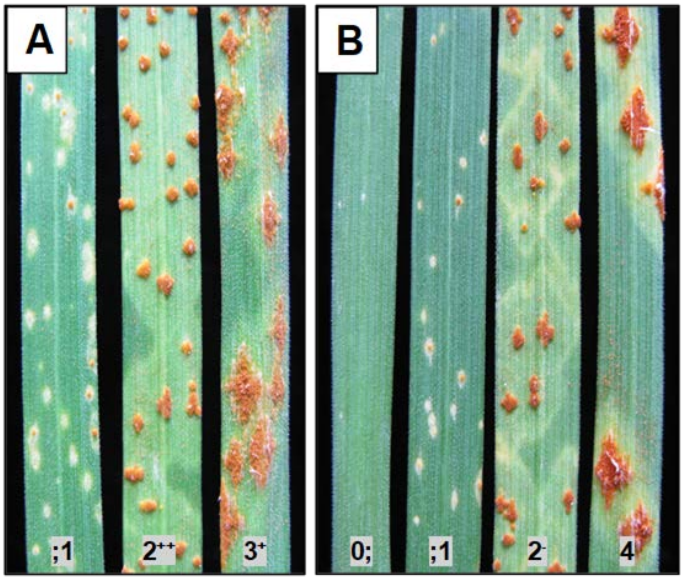

Figure 6.4: Seedling infection types representing the segregation observed in the F2 population of the cross Tobie/Coorong 12 days post inoculation with pathotype (A) UVPgt53 and (B) UVPgt56 of *Puccinia graminis* f. sp. *tritici*.

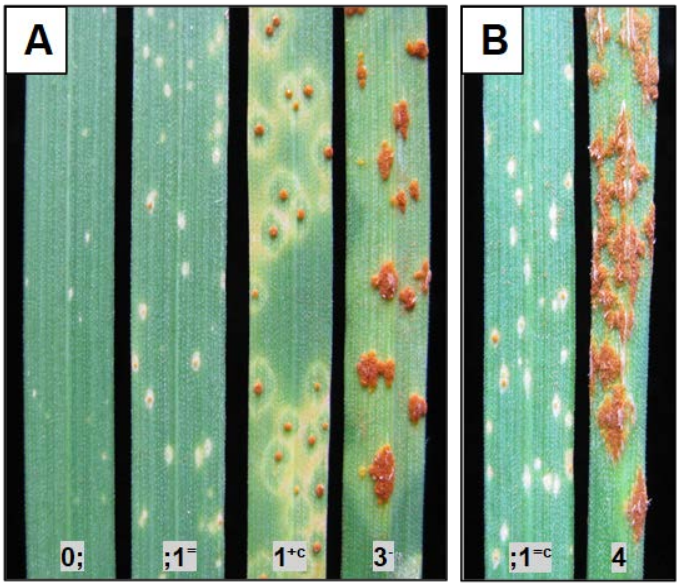

Figure 6.5: Seedling infection types representing the segregation observed in the F2 population of the cross Tobie/Kiewiet 12 days post inoculation with pathotype (A) UVPgt53 and (B) UVPgt56 of *Puccinia graminis* f. sp. *tritici*.

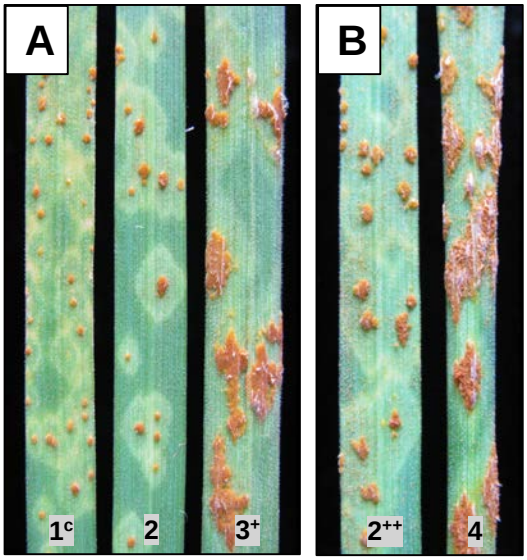

Figure 6.6: Seedling infection types representing the segregation in the F2 population of the cross Kiewiet/Coorong 12 days post inoculation with pathotype (A) UVPgt53 and (B) UVPgt56 of *Puccinia graminis* f. sp. *tritici*.

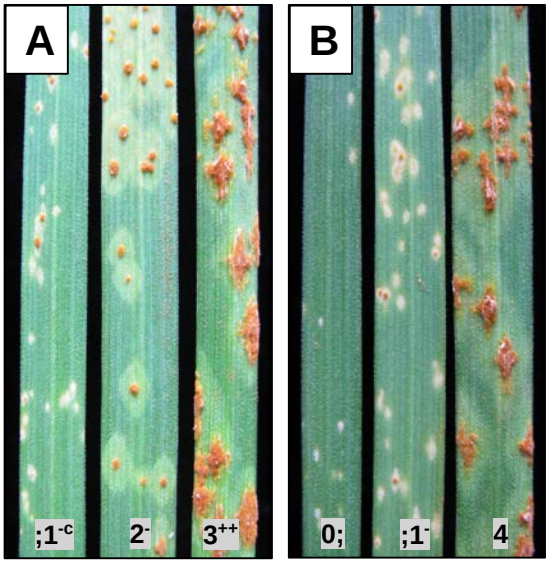

Figure 6.7: Seedling infection types representing the segregation observed in the F2 population of the cross Kiewiet/Satu 12 days post inoculation with pathotype (A) UVPgt53 and (B) UVPgt56 of *Puccinia graminis* f. sp. *tritici*.

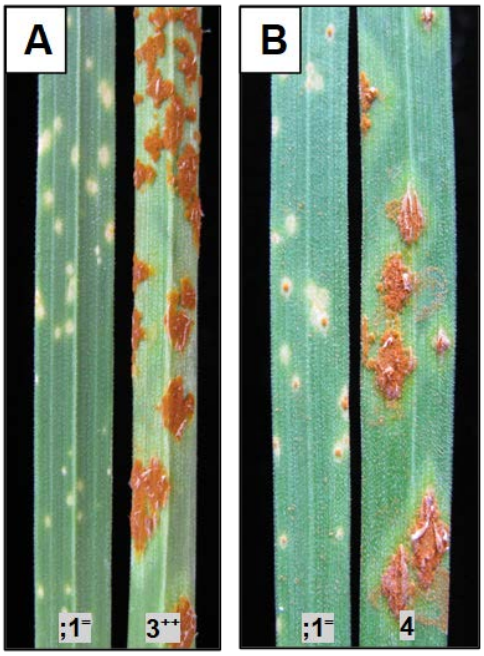

Figure 6.8: Seedling infection types representing the segregation observed in the F2 population of the cross Satu/Coorong 12 days post inoculation with pathotype (A) UVPgt53 and (B) UVPgt56 of *Puccinia graminis* f. sp. *tritici*.

Figure 6.9: Seedling infection types representing the segregation observed in the F2 population of the cross Kiewiet/SrNin twelve days post inoculation with pathotype (A) UVPgt53 and (B) UVPgt60 of *Puccinia graminis* f. sp. *tritici*.

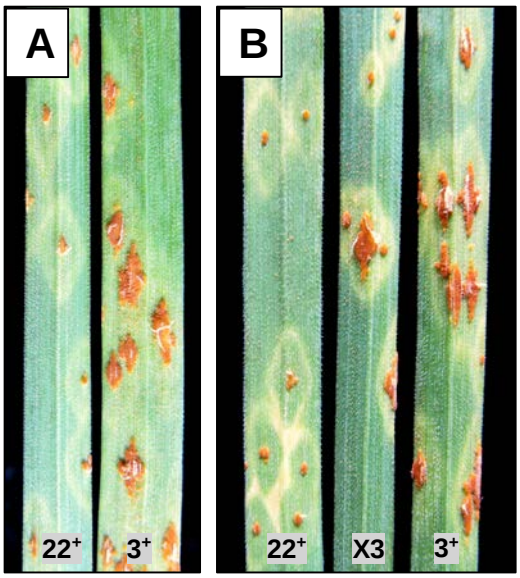

Figure 6.10: Seedling infection types for segregation observed in the F2 population of the cross Kiewiet/SrNin 12 days post inoculation with pathotype (A) UVPgt56 and (B) UVPgt57 of *Puccinia graminis* f. sp. *tritici*.

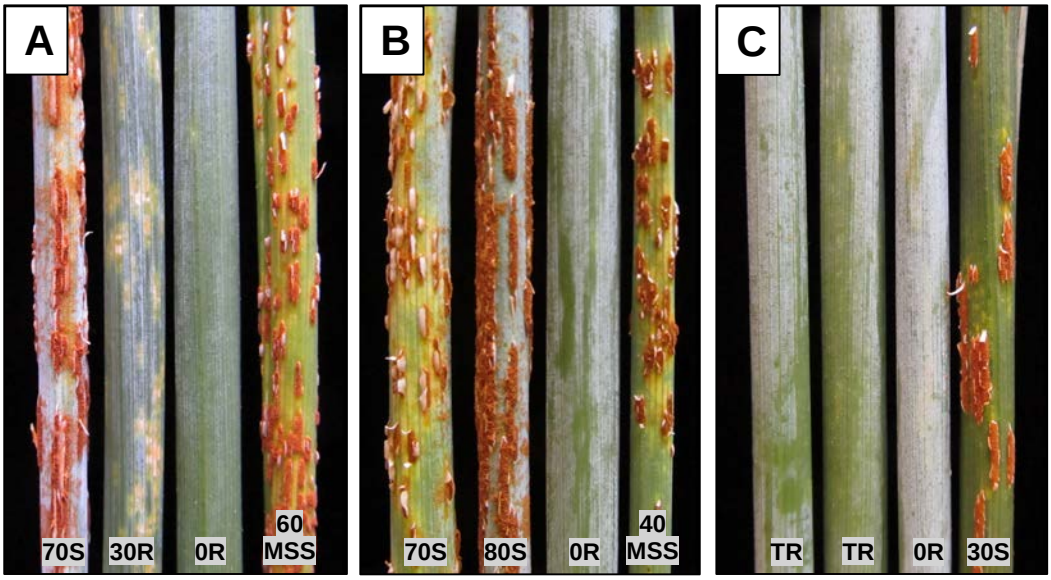

Figure 6.11: Adult plant response types on stems of parental lines Coorong, Kiewiet, Satu, and Line 37-07 (left to right) 16 days post inoculation, with pathotypes (A) UVPgt53, (B) UVPgt56 and (C) UVPgt60 of *Puccinia graminis* f. sp. *tritici*.

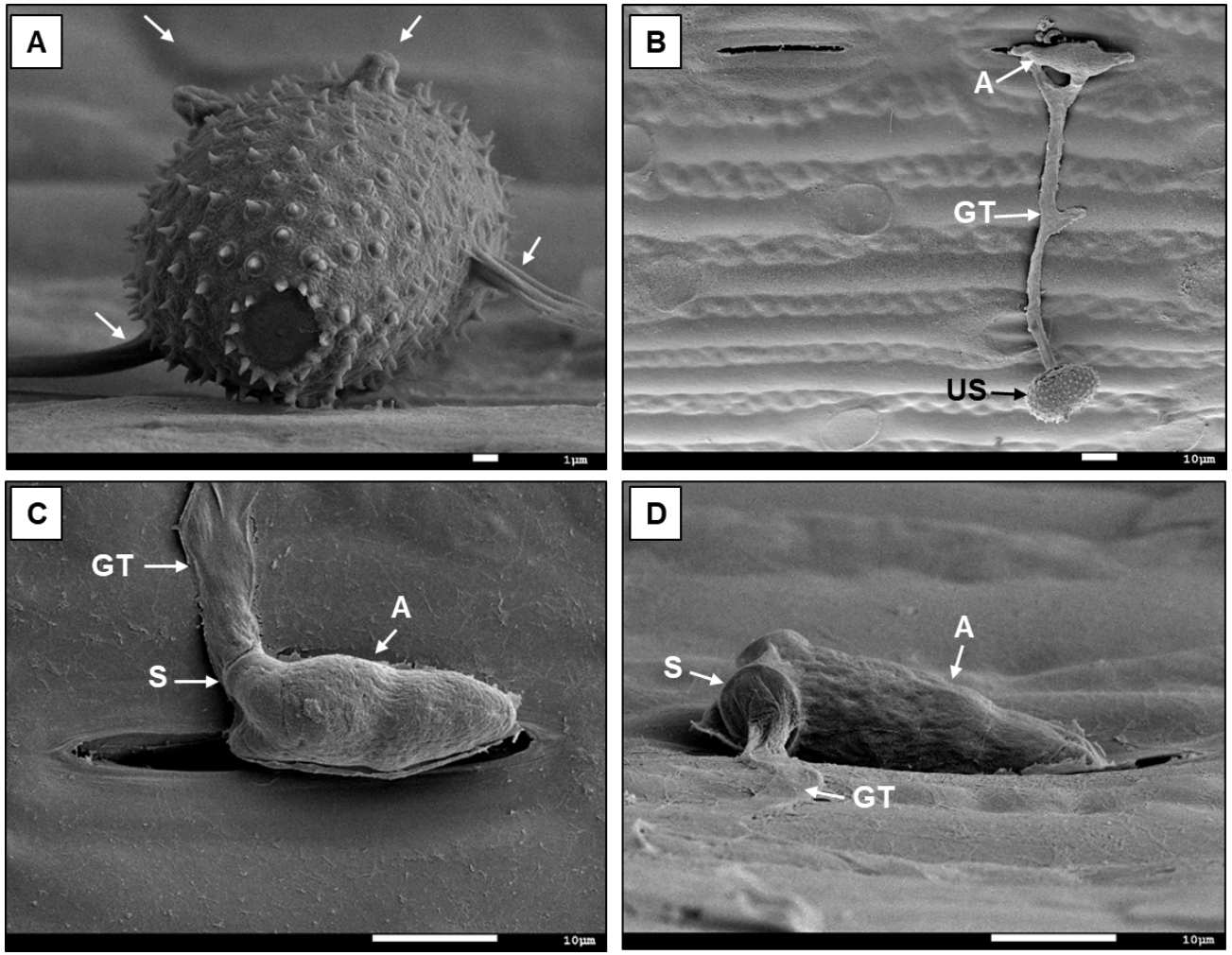

Figure 6.12: Scanning electron micrographs of (A) a germinating urediniospore (B), formation of a germtube (GT) and appressorium (A) (C), on top of a stomata and (D) the abstriction from the germtube by a septum (S) of *Puccinia graminis* f. sp. *tritici* 24 hpi on flag leaf sheaths of (A and D) Coorong and (B and C) Satu.

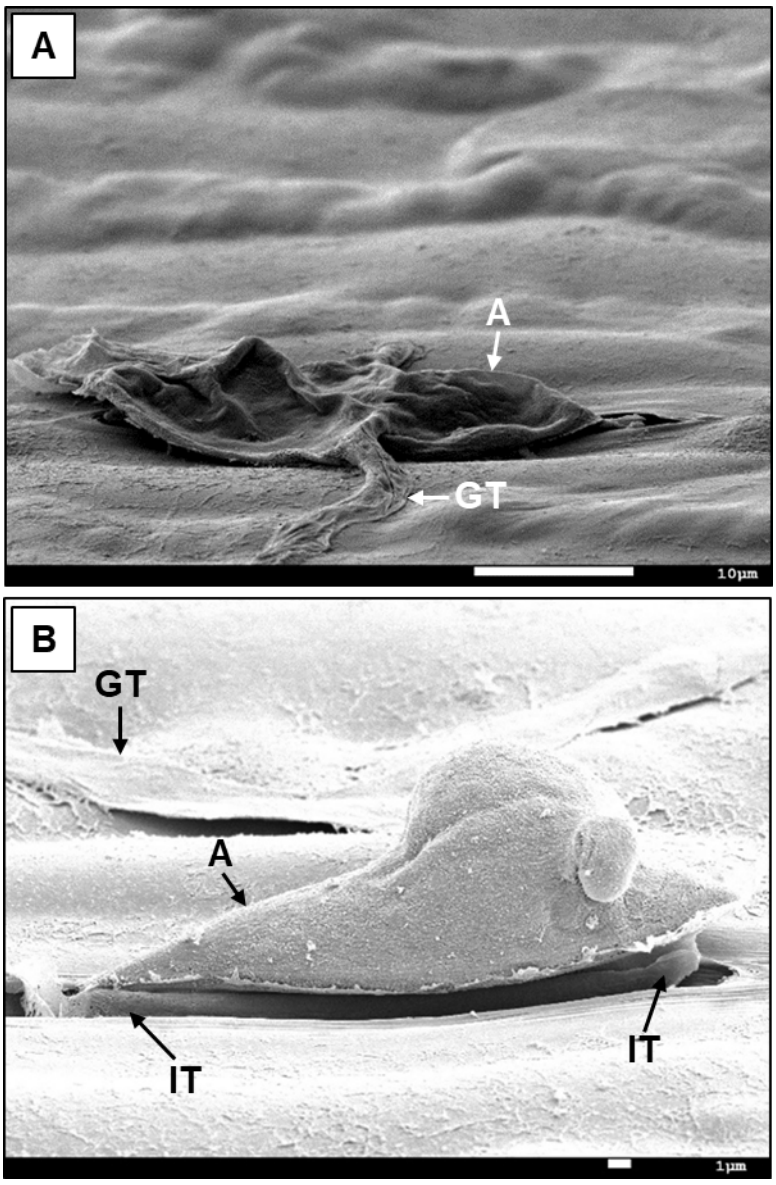

Figure 6.13: Scanning electron micrographs of (A) a collapsed appressorium (48 hpi) and (B) the interconnective tube (IT) (24 hpi) of *Puccinia graminis* f. sp. *tritici* on the outside of the flag leaf sheaths of Satu and Kiewiet respectively.

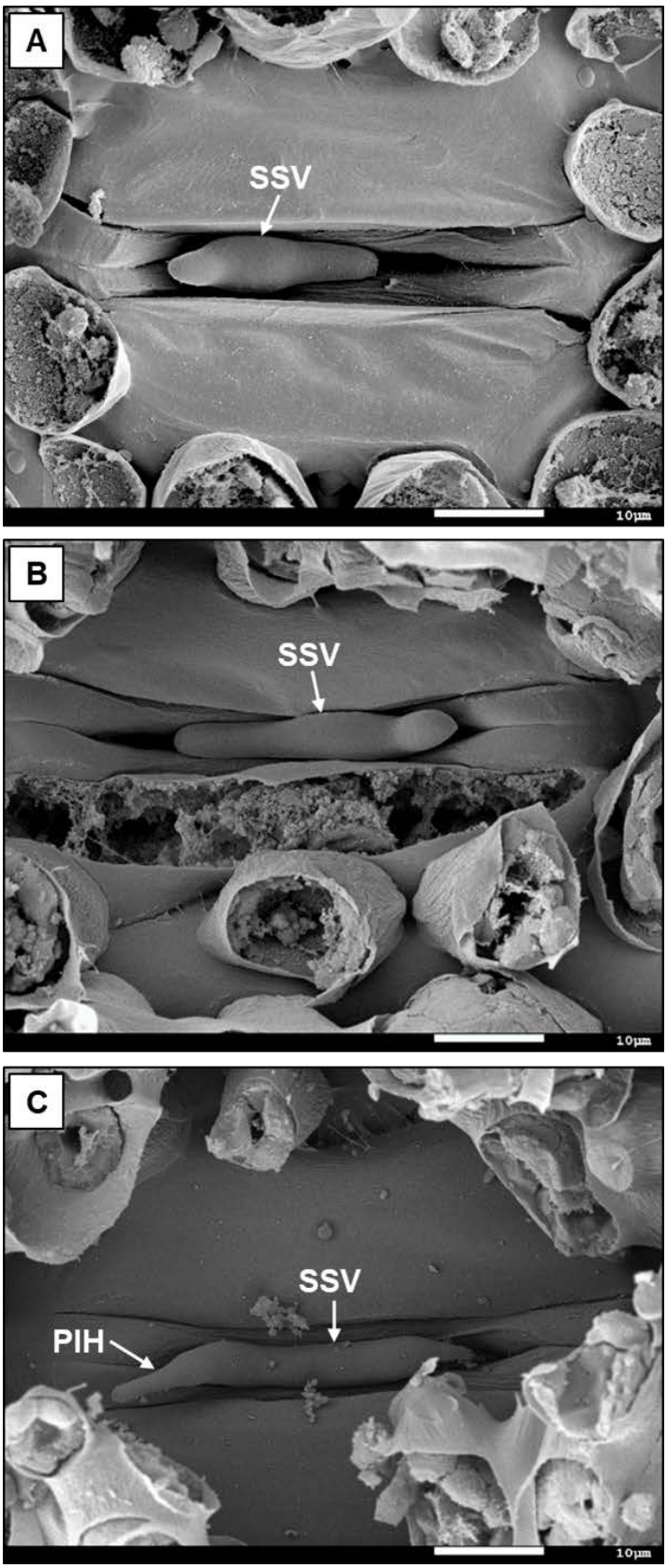

Figure 6.14: Scanning electron micrographs of substomatal vesicle (SSV) and primary infection hypha (PIH) formation of *Puccinia graminis* f. sp. *tritici* inside the substomatal cavity of the flag leaf sheaths at 24 hpi on (A) Line 37-07, (B) Satu and (C) Kiewiet.

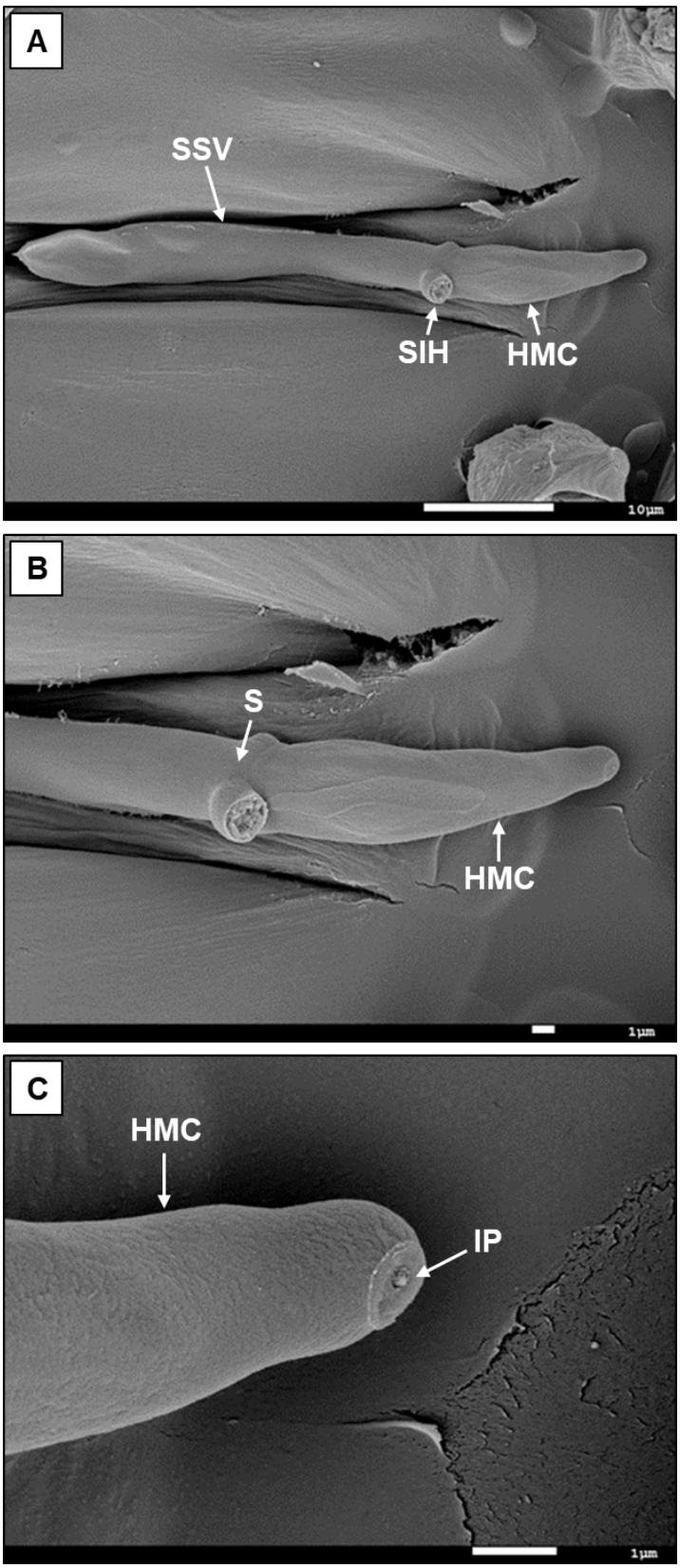

Figure 6.15: Scanning electron micrographs of the early penetration process (24 hpi) of *Puccinia graminis* f. sp. *tritici* on flag leaf sheaths of Coorong demonstrating the substomatal vesicle (SSV), the secondary infection hypha (SIH) and the formation of the haustorium mother cell (HMC) with an infection peg (IP).

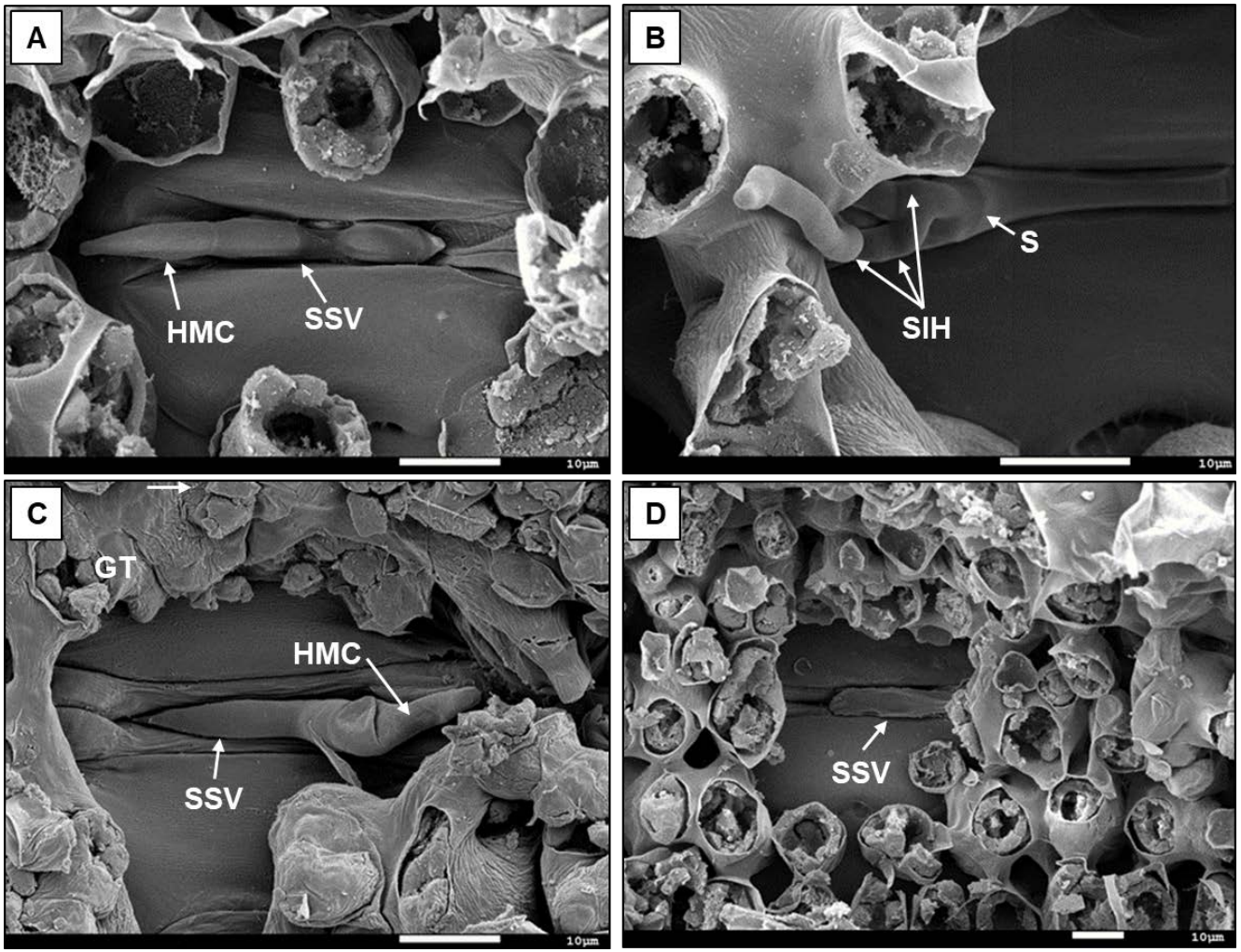

Figure 6.16: Scanning electron micrographs of *Puccinia graminis* f. sp. *tritici* development inside the substomatal cavity of the flag leaf sheaths at 48 hpi on (A) Line 37-07, (B) Coorong, (C) Kiewiet and (D) Satu. The substomatal vesicle (SSV), haustorium mother cell (HMC), septum (S) and secondary infection hyphae (SIH) are indicated.

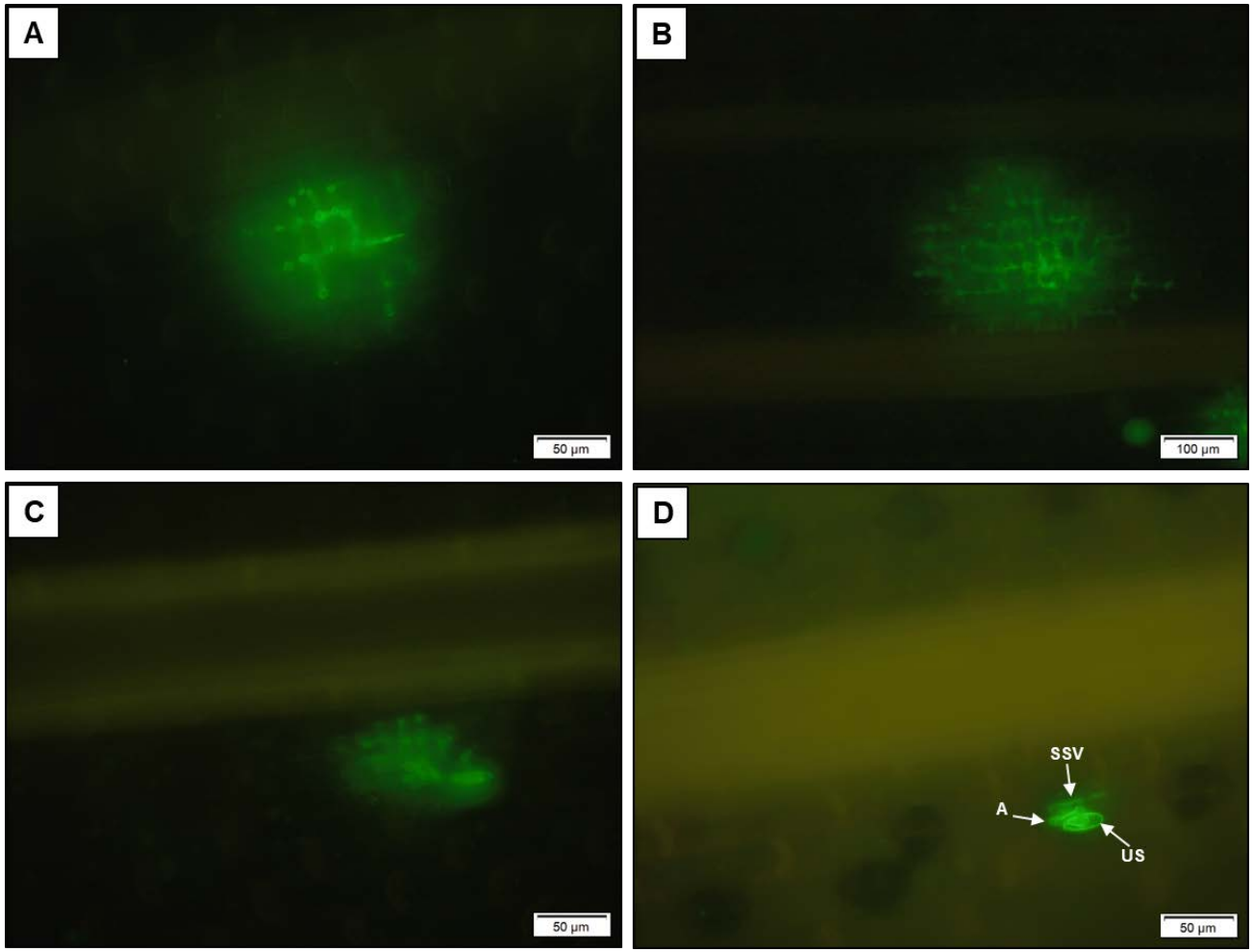

Figure 6.17: Microscope images, taken at 120 hpi, showing differences in the size of fluoresced colonies of *Puccinia graminis* f. sp. *tritici* pathotype UVPgt53 in leaf sheaths of (A) Line 37-07, (B) Coorong, (C) Kiewiet and (D) Satu. Scale bar of A, C and D represent 50 µm, whereas B is 100 µm. Urediniospore (US), appressorium (A) and substomatal vesicle (SSV) are indicated.

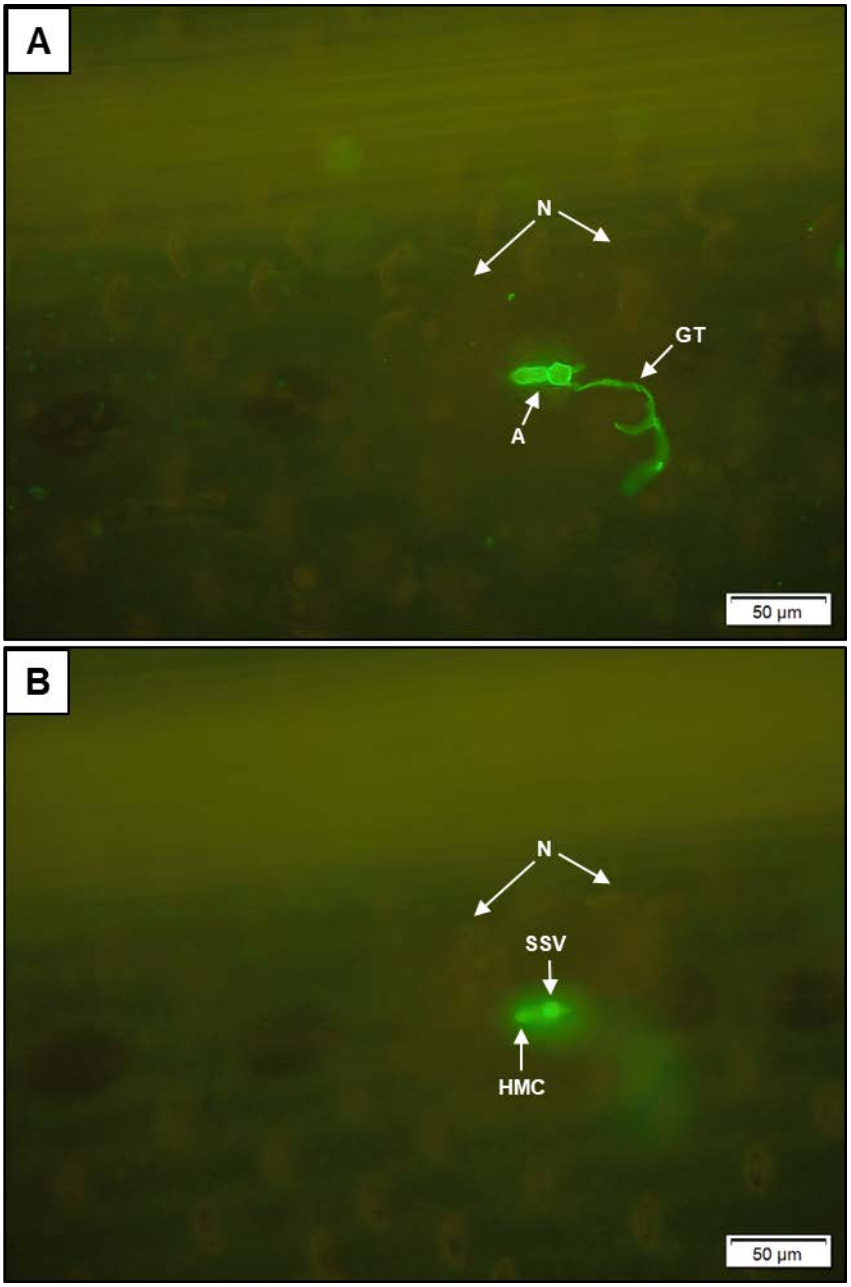

Figure 6.18: Microscope images, taken at 120 hpi, displaying the necrotic area associated with a colony of *Puccinia graminis* f. sp. *tritici* pathotype UVPgt53 in Kiewiet. (A) on top of the leaf sheath and (B) inside the leaf sheath. Scale bar represent 50 µm. The appressorium (A), germtube (GT), substomatal vesicle (SSV) and necrotic area (N) are indicated.

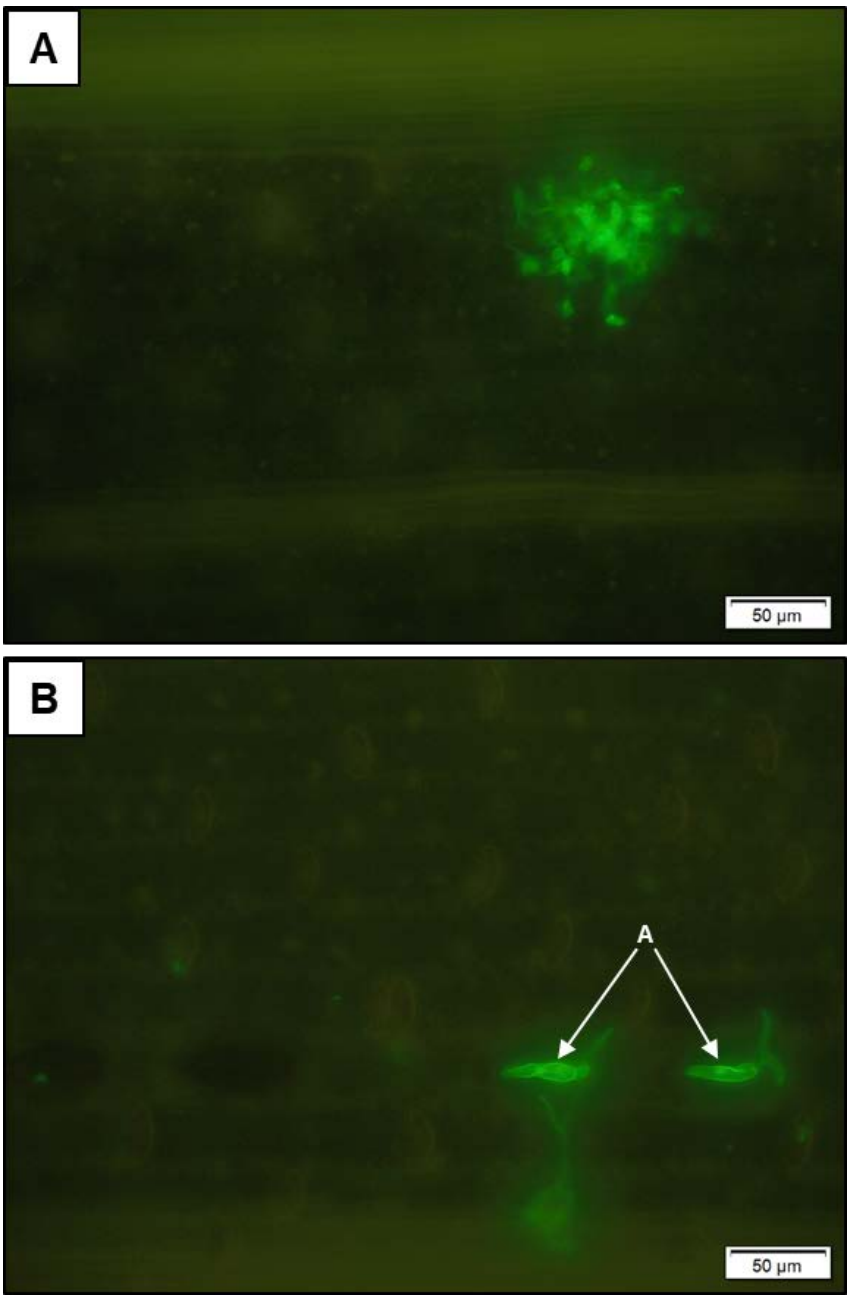

Figure 6.19: Microscope images, taken at 120 hpi, showing differences in the leaf sheath penetration process of *Puccinia graminis* f. sp. *tritici* pathotype UVPgt60 in (A) Line 37-07 and (B) Coorong. Scale bar represent 50 µm. Appressoria (A) are indicated.

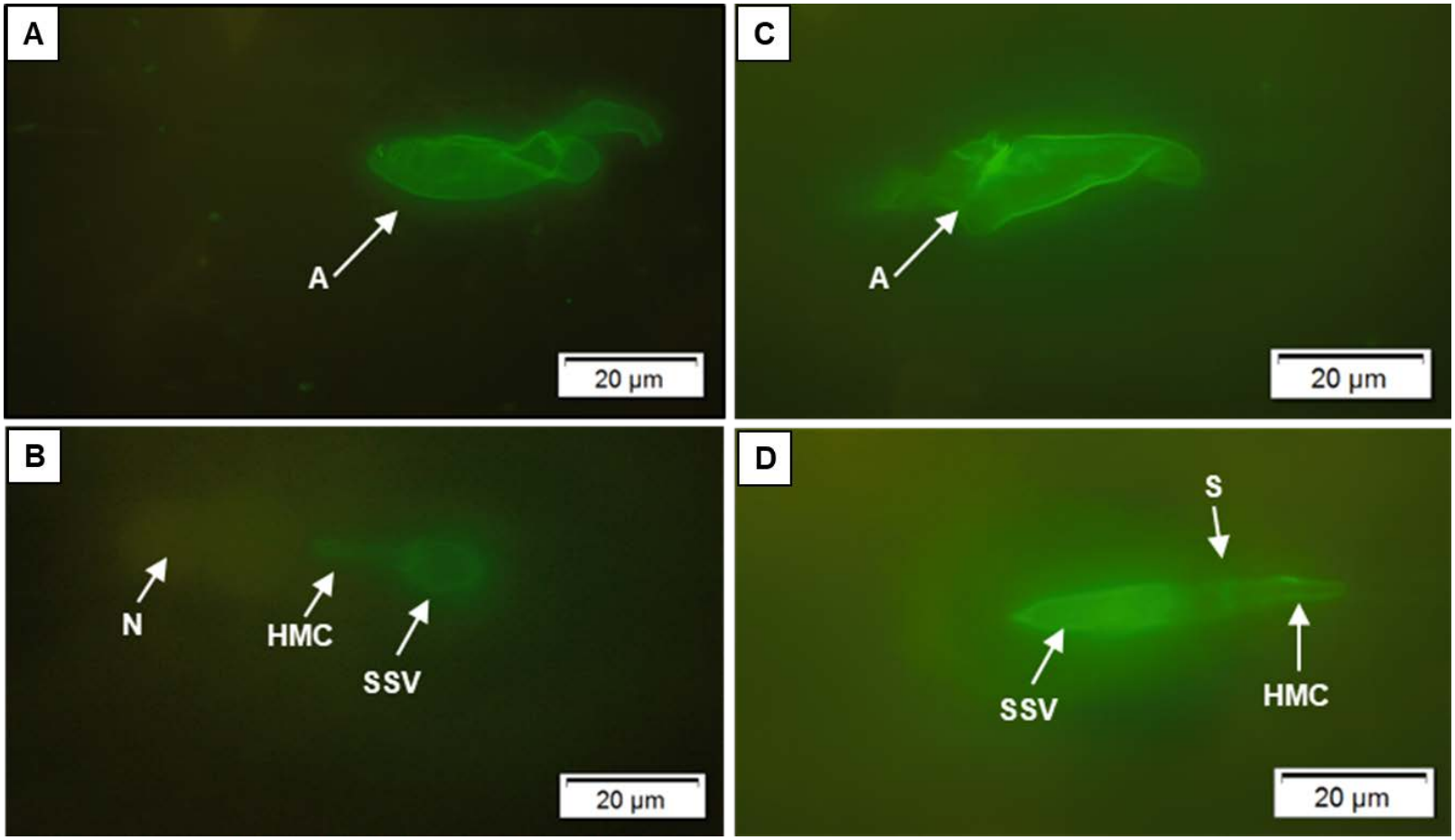

Figure 6.20: Microscope images, taken at 120 hpi, displaying the penetration process of *Puccinia graminis* f. sp. *tritici* pathotype UVPgt60 in Kiewiet on the leaf sheath surface (A) and inside the leaf sheath (B) and for Satu on the leaf sheath surface (C) and inside the leaf sheath (DB). Scale bar represent 20 µm. The appressorium (A), germtube (GT), haustorium mother cell (HMC), septum (S), substomatal vesicle (SSV) and necrotic area (N) are indicated.

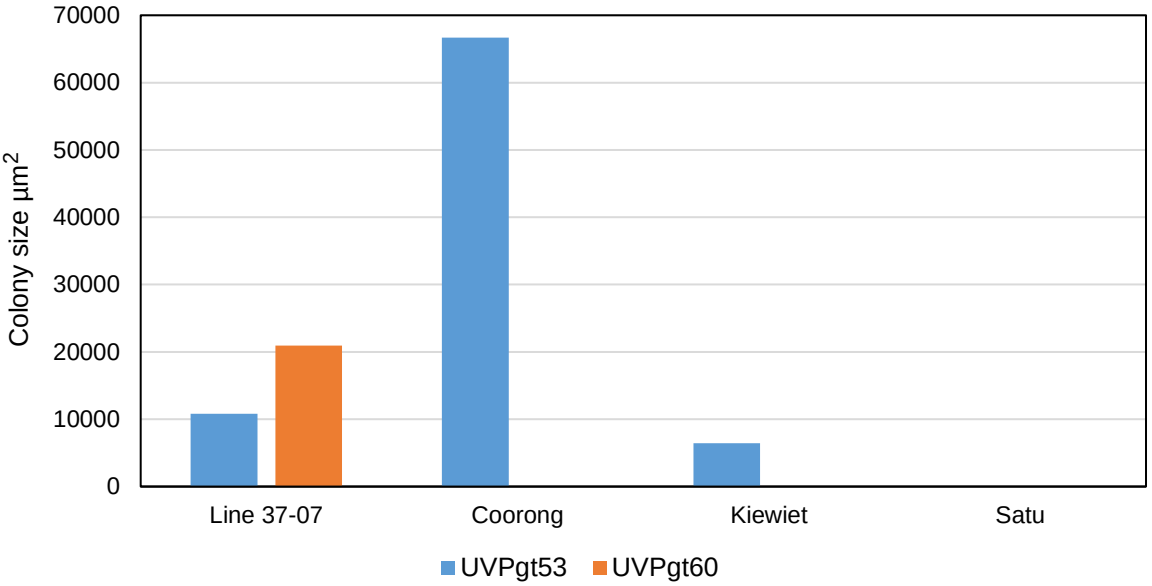

Figure 6.21: Average colony sizes (μm^2) for pathotypes UVPgt53 and UVPgt60 of *Puccinia graminis* f. sp. *tritici* measured 120 hpi in the adult plant leaf sheaths of the susceptible wheat Line 37-07 and triticales Coorong, Kiewiet and Satu. Least significant differences (LSD) among lines for colony size were calculated as 7497 ($P \leq 0.05$ ANOVA).

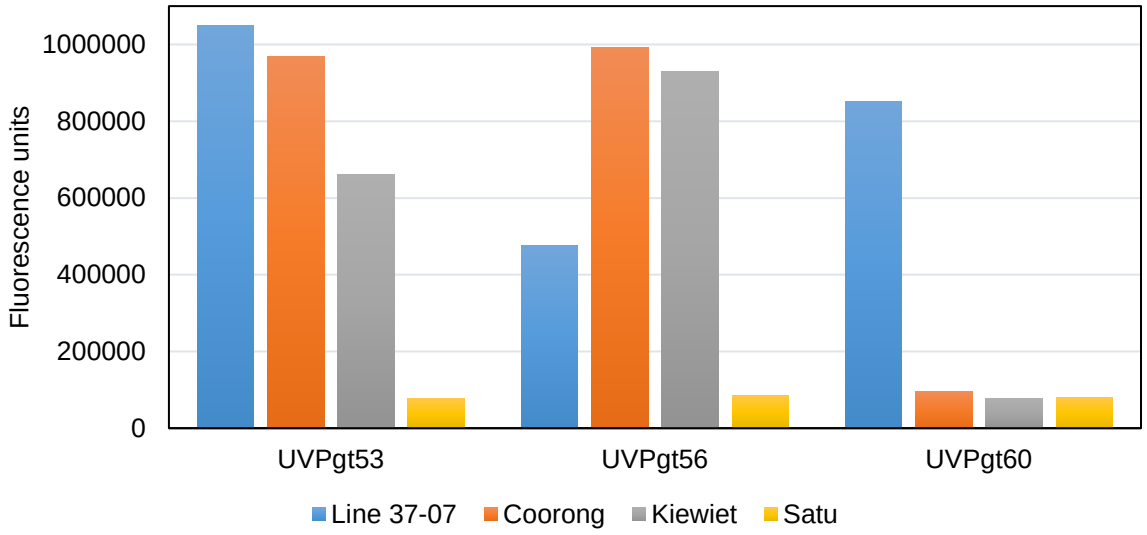

Figure 6.22: Quantification of the WGA-FITC probe (wheat germ agglutinin fluorescein isothiocyanate conjugate) bound with fungal chitin for pathotypes UVPgt53, 56 and UVPgt60 of *Puccinia graminis* f. sp. *tritici* measured 16 dpi for the susceptible control Line 37-07 and triticales Coorong, Kiewiet and Satu. Least significant differences (LSD) among lines for fluorescence measurements were calculated as 101267 ($P \leq 0.05$ ANOVA, Table 6.6).

SUMMARY

The rapid distribution of Ug99 and its variants emphasized the vulnerability of global cereal production to stem rust. Due to the evolution and mutation of *Puccinia graminis* f. sp. *tritici* (*Pgt*) pathotypes in South Africa (SA) there is a constant need to discover new sources of resistance, understand the genetic base of presently deployed sources in cereal cultivars, and to manipulate the future deployment of resistant sources through a more sustainable approach. The current study includes various fundamental aspects for effective management of stem rust in SA.

Assessment of wheat varieties elucidated a decline in seedling and adult plant stem rust resistance from 2009 to 2011. Previous studies confirmed the presence of the resistance genes *Sr24* and *Sr31* in various South African varieties and the sudden decrease in adult plant resistance (APR) observed from 2009 to 2011 after the appearance of *Pgt* pathotype UVPgt60 (PTKST) corroborated the presence of these genes in certain South African varieties. Since 2011, the withdrawal of susceptible cultivars and selection for resistance to this pathotype, resulted in an annual increase in the number of entries with seedling and APR.

In the past conventional methods of phenotyping wheat and triticale have proven to be difficult and time consuming and multi-pathotype tests were only feasible on seedlings in the greenhouse. The development of a dependable screening system for assessing APR for stem rust in the greenhouse provides an additional instrument to assess for stem rust resistance and allows for simultaneous multi-pathotype screening. The validation of the technique emphasized that it is unlikely that greenhouse phenotyping will replace field testing, especially for additively inherited resistance genes that may yield more reliable results under field conditions.

The effective management of stem rust requires race-monitoring, characterization of resistance sources and deployment thereof. A single rust species, such as *Pgt*, can be variable in its ability to attack different genera of host plants such as wheat and triticale. Although there is some understanding of rust resistance in wheat, little

is known about resistance in triticale. Inheritance studies were undertaken to determine the genetic base of certain South African cultivars through seedling analysis and field work to detect APR. Breeding emphasis should be on the stacking of resistance genes as observed for Duzi, SST 047, Steenbras and Tankwa. However, the presence of single genes detected in Tobie and Kiewiet explains the short-lived resistance to *Pgt* in both triticale cultivars. The additional seedling resistance gene expressed in Line *SrNin* needs further clarification.

To add to our understanding of the host-pathogen interaction of triticale, histological methods such as microscopy were used to elucidate the infection structures and subsequent colonization process of rust pathogens in different cereal hosts. Previously no comprehensive information existed for the infection process of *Pgt* in triticale and scanning electron microscopy results showed no evident morphological differences in pre-penetration fungal behavior when compared with wheat. Results further revealed that colony size and biomass growth are equally applicable to triticale when the expression of resistance is studied.

In conclusion, the utilization and development of different resistance screening methods, a valued study to elucidate host genetics as well as the use of histological and microscopic methods to study early resistance responses broaden our knowledge and understanding of stem rust resistance in South African wheat and triticale cultivars.