STUDIES ON CHEMICAL CONTROL OF WHEAT STEM RUST

BY

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CONTENTS

PREFACE

The reemergence of wheat stem rust as an important disease of bread wheat not only in South Africa, but also worldwide, served as justification for this study. A significant increase in stem rust occurrence in the Western Cape suggested that there is a major build-up of inoculum and that cultivars lack resistance. Although the emphasis was on chemical control, a chapter dealing with pathogenic variability in *Puccinia graminis* f. sp. *tritici* is included. Most of the survey data were obtained while the candidate was employed by the ARC Small Grain Institute at Bethlehem.

The dissertation is arranged as independent chapters and a degree of duplication was therefore unavoidable.

CHAPTER 1

AN OVERVIEW OF *PUCCINIA GRAMINIS* F. SP. *TRITICI*

1.1 INTRODUCTION

Stem rust caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., is an economically important disease of bread wheat (*Triticum aestivum* L.) worldwide (Roelfs, Singh & Saari, 1992; McIntosh, Wellings & Park, 1995). The disease has often reached epidemic proportions in South Africa, the most recent being the stem rust epidemic on *Sr24*-derived wheat cultivars in 1985 (Le Roux, 1985; Le Roux & Rijkenberg, 1987a,b).

According to McIntosh *et al*. (1995), effective genetic control of rust diseases requires a coordinated effort, including pathotype monitoring, collection and characterization of sources of resistance, and resistance breeding. Data generated by pathotype surveys thus form an essential component of breeding programmes and are conducted by most wheat producing countries, principally to recognize pathogenic changes (McIntosh *et al*., 1995). The timely detection of stem rust pathotypes with new virulence is considered important to the South African wheat industry.

The effect of a shift in virulence on cultivar susceptibility, and thus potential losses, is important to producers aiming for maximum yields. Similarly information on pathogenicity enables geneticists to utilise the most effective resistance genes in their breeding programmes. In South Africa, 22 pathotypes of *P. graminis* f. sp. *tritici* that occur on wheat and triticale (X *Triticosecale* Wittmack) have been characterised from 1981-1997 (Le Roux & Rijkenberg, 1987b; Le Roux, 1989; Smith & Le Roux, 1992; Boshoff, Van Niekerk & Pretorius, 2000).

A recent review of *P. graminis*, including the wheat stem rust pathogen, focused on the importance, taxonomy, host range, symptoms and genetics of this fungal species (Leonard & Szabo, 2005). The objective of the present overview is to provide a summary of the wheat stem rust pathogen, its symptoms, epidemiology and disease control.

1.2 PATHOGEN

Stem rust of wheat is caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. The disease is also known as black rust or summer rust (Roelfs, 1985). Stem rust is an obligate parasite that can only grow in living host material (Wiese, 1987). This particular *forma speciales* can infect numerous wheat cultivars but also a few barleys, rye and some grasses (Knott, 1989). Apart from wheat and its alternate hosts, the stem rust fungus has a relatively narrow host range, but some grasses are important sources of primary inoculum (Wiese, 1987).

In addition to wheat, the fungus completes part of its life cycle on alternate hosts, especially barberries (*Berberis vulgaris* L. and *B. canadensis* Mill.) and certain species of *Mahonia* (Roelfs, 1985). In 1916, Stakman found that *P. graminis* f. sp. *tritici* comprises different physiological types (races), and that certain wheat varieties while resistant to some races, were fully susceptible to others. Numerous specified pathotypes of *P. graminis* occur and are probably formed by mutation or somatic hybridization (Luig, 1977). Burdon *et al.* (1982) used isozyme phenotypes to assess the origin and evolution of stem rust in Australia and found that the major changes in the wheat stem rust flora resulted from exotic introductions.

1.2.1. Host specialisation

Within *P. graminis,* specialization on particular host genera has occurred to produce *formae speciales*. Five physiological varieties, some of which consist of a number of races are *P. graminis* f. sp. *avenae* on oat and related grasses, *P. graminis* f. sp. *secalis* on rye and related grasses, *P. graminis* f. sp. *agrostidis* on *Agrostis* spp., *P. graminis* f. sp. *poae* on *Poae* spp. and most importantly, *P. graminis* f. sp. *tritici* on wheat, barley and many of the relatives of wheat (Verwoerd, 1935; Knott, 1989).

Many species of *Berberis, Mahonia,* and their hybrid (X *Mahoberberis*) are susceptible to *P. graminis.* According to Le Roux & Rijkenberg (1987a), stem rust oversummers on grasses in South Africa namely *Hordeum murinum* Huds. and *Festuca arundinacea*. Pretorius *et al*. (2007) reviewed literature describing *Agropyron distichum, Hordeum vulgare, Lolium italicum, Bromus maximus* and *Dactylis glomerata* as accessory hosts in South Africa. Infection with *P. graminis* f. sp. *tritici* has been found on 112 species of wild and cultivated grasses from Israel (Gerechter-Amitai & Wahl, 1966).

1.2.2. Pathotype differentiation

The use of race–specific resistance to control wheat stem rust requires continued monitoring of the variation in the pathogen population for virulence (Roelfs, 1985). In 1916, Stakman and colleagues at St. Paul, Minnesota, U.S.A., showed that *P. graminis* f. sp. *tritici* comprised different biotypes. This discovery revolutionised the science of plant pathology and different biotypes were subsequently discovered in many other pathogens (Luig, 1983). These variants have been termed races, physiologic forms, pathotypes and strains.

Several systems are or have been used for the identification of physiological races in *P. graminis* f. sp. *tritici*.

- International system (Stakman, Stewart & Loegering, 1962).
  Twelve standard differentials were used worldwide until about 1950. This set is still in use for communication purpose worldwide. Physiologic races are based on combinations of host reaction classes.
- Modified potato – *Phytophthora infestans* system (Watson & Luig, 1962).
  Nomenclature consists of the international race number, followed by ANZ for New Zealand and then the numbers of the additional differential hosts that are susceptible. According to McIntosh *et al.* (1995) a selection of six genotypes, an additional 11 wheats and two triticale lines, are used as a supplementary differential set in Australia.
- Formula-method (Green, 1965).
  Used in Canada to identify physiologic races according to the avirulence / virulence formula for each pathotype.
- Coded sets (Roelfs & Martens, 1988; Roelfs, Long & Roberts, 1993).
  Four sets of four host lines each with a single gene for resistance are used to identify physiologic races in the U.S.A and Canada. New subsets can be added without affecting the previously used coding. Differential hosts are placed in sets of four, and a letter is assigned to each of the possible resistant/susceptible combinations.
- South African system (Pretorius *et al.*, 2007).
  New races are identified by an alpha-numeric code. The identity consists of three components. A number denote the rust type viz. 2 denote *P. graminis* f.

sp. *tritici* and 3 *Puccinia triticina*, followed by SA and an accession number for each distinctive pathotype.

1.2.3. Prevalence of virulence in *Puccinia graminis* f. sp. *tritici* worldwide

Worldwide virulence for *Sr2, 13, 22, 24, 25, 26, 27, 29, 31, 32, 33, 34, 37, Gt* and *Wld-1* is absent or limited. *Sr13* is ineffective at lower temperatures, whereas *Sr29* and *34* may be ineffective under high inoculum densities (Roelfs *et al.*, 1992). Virulence for *Sr24* and *Sr27* exists in South Africa (Le Roux & Rijkenberg, 1987a). *Sr26* virulence is undetected despite the widespread use of the cultivar Eagle and its derivatives in Australia (Luig, 1983; McIntosh *et al*., 1995). The extensive use of *Sr31* in Kavkaz and similar wheats with the 1B/1R translocation did not result in virulence for this gene worldwide until *Sr31* succumbed in Uganda in 1999 (Pretorius *et al.*, 2000).

Virulence for *Sr6, 11* and *17* is common wherever these genes have been used. Virulence for *Sr5, 9e* and *21* appears to be common in some areas, but remains low or absent in other areas. Virulence is common for *Sr8b* (except in Australia, New Zealand and South Africa), *Sr9a, Sr9d* and *Sr14* (except North America), *Sr12* (except North America, Australia and New Zealand), *Sr15* (except Africa, North America, Australia and New Zealand), and for *Sr16, Sr18, Sr19, Sr20* and *Sr28* (except in China, India, Nepal, Pakistan and Ethiopia) (McIntosh *et al.*, 1995). Table 1 contains references to published virulence surveys. Luig (1983) summarised the distribution of wheat stem rust races from 1955 through 1966 on a worldwide basis and Green (1975) determined the evolution of virulence combinations in Canada.

1.2.4. History of wheat stem rust in South Africa

The history of South Africa is rich in examples of the battle between the wheat industry and stem rust (Pienaar, 1975). Commercial production of wheat in South Africa dates back to 1658 just after the Dutch settlement in 1652 (Lombard, 1986). At the turn of the previous century Dr. E.A. Nobbs, agricultural advisor at the Cape, made the first crosses at the Robertson Experimental Station to improve the milling quality of South African wheat. In 1891, twelve wheat cultivars where imported from England together with the local cultivar ‘Du Toits.’ Ten of these cultivars were ‘rust-proof’ in England, but were severely damaged in trials at Somerset East (Lombard, 1986). The first cultivars bred in South Africa were Union, Darlvan and Nobbs, which

were released in 1910 (Pienaar, 1975). In 1925, the cultivars Bobriet and Gluretty were released by Neethling with stem rust resistance derived from the Italian cultivar Rieti (Pienaar, 1975). In 1948, the cultivar Hoopvol was released because its horizontal resistance to stem rust has withstood the test of time (Pienaar, 1975). In 1930 several physiological forms of *P. graminis* f. sp. *tritici* were recorded (Verwoerd, 1935).

The first major stem rust epidemic occurred in 1726, when the entire crop was lost (Lombard, 1986). It has been suggested that urediniospores were carried and dispersed with the cereal and grass hay that accompanied livestock on ships en route to South Africa. When urediniospores are well dried and protected from temperature and humidity variations, they can remain viable for a long time (Lombard, 1986). A well-documented epidemic occurred in 1957/1958, when winter wheat production in the Free State was devastated by rust (Lombard, 1986). A potential epidemic threatened in the 1980's due to the over-utilisation and geographically wide deployment of resistance gene *Sr24* in South Africa (Le Roux & Rijkenberg, 1987a). Severe losses eventually occurred on *Sr24* cultivars, particularly on wheat production in the Cape.

In 1980, the Department of Agriculture decided to make stem rust resistance mandatory for all released cultivars and it became imperative to conduct annual pathotype surveys (Le Roux & Rijkenberg, 1987b). Stem rust pathotypes recorded in South Africa between 1935 and 2000 are documented in Table 2.

1.3 SYMPTOMS

Raised orange-red pustules occur on leaves, leaf sheaths, stems and occasionally on glumes, awns and even seed of susceptible wheat cultivars (Scott, 1990). Figure 1 shows the orange-red pustules on the stem. Uredinial pustules are conspicuously erumpent, with shredded epidermal tissues at their margins. They may erupt through both leaf surfaces but tend to be larger on the lower surface (Wiese, 1987). The pustules are oval, elongated or spindle-shaped and up to 3 X 10 mm in size (Roelfs *et al.*, 1992). Urediniospores are 15-24 x 21-40 µm in size, orange-red, dehiscent and oval, oblong or ellipsoid. Four median germ pores indent their thick, spiny walls (Wiese, 1987).

Teliospores are formed later in the season and the pustules become almost black in colour (Scott, 1990). Teliospores are ellipsoid to clavate, 15-20 x 40-60 µm in size and two-celled. They are tapered at their apex and have smooth, thick walls (Wiese, 1987).

Germination of the teliospore normally follows after several weeks of cold dormancy and yields a hyaline basidium (promycelium) on which basidiospores develop on sterigmata (Wiese, 1987). The basidiospores are small, 7.6 x 6 µm, hyaline and oval-shaped. They are windborne and are spread to alternate hosts such as *Berberis* spp. (Roelfs, 1985).

Pycnia on barberry are small, flask-shaped, sunken and the supporting leaf tissues are typically discoloured yellow-red. Pycnia produce pycniospores (1.6 x 3.6 µm) in small sticky droplets that attract insects (Roelfs, 1985).

Aecia on the underside of barberry leaves are yellow and hornlike, projecting up to 5 mm from the leaf surface. Aeciospores produced by aecia in long dry chains are subglobose, 15-19 x 16-23 µm, smooth and light orange-yellow (Roelfs, 1985). Neither *Berberis vulgaris* nor the aecial stage of *P. graminis* f. sp. *tritici* is known to occur in South Africa.

1.4 EPIDEMIOLOGY

1.4.1. Life cycle

In most areas of the world, the life cycle (Fig. 2) of *P. graminis* f. sp. *tritici* consists of continual uredinial generations. The fungus spreads by airborne urediniospores from one wheat plant to another and from field to field (Roelfs *et al.*, 1992). Primary inoculum may originate locally (endemic) from volunteer plants or be carried long distances (exodemic) by wind and deposited by rain (Wiese, 1987). In certain areas snow can provide cover that occasionally permits the fungus to survive on winter wheat even at subzero temperatures (Roelfs & Long, 1987). Urediniospores germinate and produce a germ tube and an appressorium. Light stimulates the formation of a penetration peg that enters a closed stoma (Staples & Macko, 1984). The repeating asexual cycle then involves urediniospores producing uredinia in about a 14-day cycle under optimum conditions (Joshi & Palmer, 1973).

The sexual cycle seldom occurs except in certain regions of the United States. Although the sexual stage gives rise to genetic diversity, it also produces large numbers of individuals that are less fit (Roelfs & Groth, 1980). As the host matures, telia are produced directly from urediniospore infections or teliospores can be produced in mature uredinial pustules.

Basidiospores germinate and penetrate barberry leaves directly. Infection results in the production of pycnia and formation of pycniospores. Pycniospores must mate to produce aeciospores (Luig & Watson, 1972). Aeciospores are hydroscopically released from the aecium and are airborne to wheat over distances of meters to perhaps a few kilometres. Aeciospores require similar conditions for infection to that of urediniospores. Infection by aeciospores results in the production of uredinia with urediniospores (Roelfs *et al.,* 1992).

Wind frequently transports urediniospores up to 100 km and sometimes up to 2000 km (Roelfs, 1985). Stem rust urediniospores are rather resistant to atmospheric conditions if their moisture content is moderate (20-30%). Long distance transport occurs annually 800 km across the North American Great Plains, nearly annually 2000 km from Australia to New Zealand, and at least three times in the past 75 years 8000 km from East Africa to Australia (Roelfs *et al.,* 1992).

1.4.2. Inoculum sources

In areas where no alternate host is found, e.g. Australia, Argentina and South Africa, stem rust oversummers on grasses or volunteer wheat. The amount of oversummering rust depends on the number of volunteer plants, the alternate hosts and the ability of urediniospores to travel over long distances (Roelfs, 1985). Sporulating uredinia are active in tropical and some subtropical areas throughout the winter. Occasional dormant mycelium may survive beneath the snow pack in northern temperate regions (Knott, 1989). In the northern hemisphere spring-sown wheat is particularly vulnerable in the higher latitudes if sources of inoculum are located downwind. Large areas of autumn-sown wheat occur in the southern American Great Plains, providing inoculum for the northern spring-sown wheat crop (Roelfs *et al.,* 1992). According to Luig & Watson (1977) population shifts in the pathogen are mainly attributed to factors other than survival ability on grasses. In terms of epidemic potential it should be noted that a stem rust pustule can produce 10 000 urediniospores per day (Roelfs *et al.,* 1992).

1.4.3. Environmental conditions

The development, extent and severity to which rust develops are, apart from host and pathogenicity factors, strongly influenced by the environment. Suitable temperature and moisture conditions, as well as a susceptible host, are necessary to ensure the development of the different spore types. In addition, the direction and velocity of winds ensure their rapid dissemination and distribution (Staples & Macko, 1984). The development and nature of rust are dependent on:

- Abundant viable inoculum.
- Rapid distribution of such inoculum.
- Favourable climatic conditions, e.g. sufficient moisture (from rains, mist or dews) for germination, infection and rapid development of successive cycles of urediniospores.
- Dense and widespread population of susceptible hosts.
- The stage of growth of the susceptible host.

High temperatures and abundant moisture (especially light drizzles, heavy mists or dews) favour rapid rust development while cool, dry weather tends to retard such development. The minimum, optimum and maximum temperatures for spore germination are 2°C, 15-24°C and 30°C and for sporulation, 5°C, 30°C and 40°C, respectively (Hogg *et al.*, 1969). According to Knights & Lucas (1980), fully dehydrated spores failed to germinate and there is a positive correlation between hydration time and percentage spore germination.

Stem rust is more important late in the growing period, on late-sown and maturing wheat cultivars and at lower altitudes. In warm, humid climates, stem rust can be especially severe due to the long period of favourable conditions for disease development when a local inoculum source is available (Wiese, 1987). Stem rust requires a relatively long dew period (6 to 8 h). Maximum infections are obtained with 8-12 h of dew at 18°C followed by 120 µE/m^2/s light while the dew slowly dries and the temperature rises to 30°C (Rowell, 1984).

Fluctuations in moisture conditions or paucity of viable inoculum may result in a light epidemic or confine the development of rust to small areas or patches in a field. The occurrence of suitable climatic conditions will determine the subsequent development and extension of rust from such centres (Roelfs *et al.*, 1992).

Rust severity may be further increased by excessive nitrogen fertilization resulting in denser stands and delayed maturity (Knott, 1989). The locality and nature of soil conditions may be a factor only in as far as it affects moisture and temperature conditions and the period of growth of the cereal host plant (Staples & Macko, 1984).

1.5 ECONOMIC IMPORTANCE

The damage caused by wheat stem rust can be more spectacular than any other cereal disease. Millions of hectares of an apparently healthy crop with a high yield potential can be totally destroyed in less than a month (Roelfs, 1985). Stem rust is a destructive disease in most wheat regions of the world. Its severity globally is well documented in published information from India, China, South Africa, Asia, America Europe and East Africa (Harder, Mathenge & Mwaura, 1972; Bahadur, 1985; Knott, 1989; Roelfs *et al.,* 1992). The fear of stem rust is understandable because a crop could be reduced to broken stems and shrivelled grain by the time of harvest (Roelfs *et al.,* 1992).

The four basic components of grain yield in wheat are the number of heads or productive tillers per square meter, the number of kernels per head, the number of kernels per spikelet, and the mass of individual kernels (Teng & Gaunt, 1980). The number of tillers and spikelets per tiller are determined before booting, the kernels per spikelet from stem elongation to the early milk stage, and the kernel mass after anthesis. Therefore, stress factors influencing the plant at different growth stages affect the respective yield components differentially (Teng & Gaunt, 1980). Lesions of rust can occupy a significant portion of the host plant tissue. Most of the sites where infection occur are sources of nutrients that are transported to the developing grain (Wiese, 1987).

Stem rust is the most devastating of the rust diseases and can cause losses of 50% in one month when conditions for its development are favourable. Losses of 100% can occur on susceptible cultivars (Roelfs *et al.,* 1992). When stem rust is extremely severe on a portion of the stem, the straw may break or lodge.

In the U.S.A., stem rust epidemics have occurred over large areas on the Central plains. It was eventually controlled by the development and production of resistant cultivars. Windborne urediniospores, nevertheless, resulted in devastating epidemics of stem rust on wheat in 1935, 1937, 1953 and 1954 and the last major

epidemic occurred in 1954 (Roelfs & Martens, 1988). In 1954, a combined epidemic of leaf and stem rust probably caused losses of more than $500,000,000 in Canada and the U.S.A. (Knott, 1989). Green and Campbell (1979) estimated the annual savings from growing rust-resistant cultivars on the Canadian prairies to be $217 million.

During the years 1930, 1931, 1948 and 1955, epidemics of stem rust of wheat caused significant losses of crops in Greece (Skorda, 1966). Rees & Syme (1981) reported that severe stem rust epidemics occurred on the cultivar Oxley, while grain yields reduced by 50%. Despite rapid changes in virulence in the pathogen, damage has been minimised by the timely release of resistant cultivars. On three occasions there have been sudden changes in rust races in Australia that appear to have originated from Southern Africa (Luig, 1983).

In Australia severe stem rust epidemics in the 1880's resulted in sufficient public concern and political pressure to combat the disease. Crop losses ranged from 30% to 55% in wheats susceptible to both stem and leaf rust. In 1973, national losses due to stem rust ranged from $A100-200 million (McIntosh *et al.,* 1995). In a study done by Pretorius (1983), the effect of stem rust on the reduction of 1000 kernel mass and plot yield was between 7% and 35% depending on the cultivar and environmental conditions.

1.5.1. Global threat of stem rust pathotype Ug99

The deployment of the 1BL.1RS translocation, containing the *Sr31* gene, provided the main component for stem rust resistance in many wheats for more than 30 years (Wanyera *et al.*, 2006). Recently, isolates of *P. graminis* f. sp. *tritici* with virulence to *Sr31* (pathotype Ug99) were detected in Uganda in 1999 (Pretorius *et al.*, 2000). The detection of the new pathotype poses a major threat to most of the wheat production areas of the world. According to surveys, virulence for *Sr31* is now widespread in the Eastern African highlands (Wanyera *et al.*, 2006). During 2001, the cultivar Shinna became highly susceptible to stem rust in Ethiopia as well as the two major cultivars grown (56% of small scale production). In 2002 the yield losses in Kenya were estimated as much as 71% under experimental conditions (Global Rust Initiative, 2005).

During January 2007, pathologists announced that Ug99 had crossed the Red Sea into the Arabian Peninsula and that it now threatens the major wheat production

areas of Asia (Stokstad, 2007). About 70% of U.S. wheat varieties are thought to be susceptible to Ug99. Between 70 and 75% of wheat grown in India and Pakistan are also susceptible and wheat in Egypt and China is thought to have similar vulnerabilities (Jin & Singh, 2006).

1.6 DISEASE CONTROL

Plant disease control strategies should be directed at reducing the probability of epidemics, as well as reducing the magnitude of losses. Integrated cereal rust management, including cultural control practices, genetic resistance and fungicide applications, should contribute to the successful control of stem rust. Due to the airborne nature of stem rust spores, quarantine measures usually delay, but do not prevent entry of pathotypes with specific virulence combinations into new areas (Roelfs, 1985; Knott, 1989 and Roelfs *et al.*, 1992). The profitability of any crop protection strategy depends on the prevalence of the disease, eventual yield loss, cost and efficacy of control measures (Fabre, Plantegenest & Yeun, 2007).

1.6.1. Cultural practices

Cultural practices provide an alternative measure for reducing the risk of wheat rust epidemics, but no single practice is effective under all conditions and a series of activities are necessary for effective control (Roelfs *et al.*, 1992). Cultural methods focus on early maturing cultivars and early planting of spring wheat (Roelfs, 1985). Delaying planting and the removal of volunteer grasses a few weeks before planting may prevent early infections and reduce the primary inoculum (Knott, 1989). Whatever the situation, each cultural practice must be tested against the anticipated types of epidemic that occur in the area (Roelfs *et al.*, 1992)

Dill-Macky & Roelfs (2000) found that reduced stand densities may promote the development of stem rust in barley. High levels of stem rust occasionally occurred in commercial fields where sparse stands are encountered. Diversity in the cultivars grown on a farm and spacing between fields can provide substantial benefits. On large farms, it may help if fields are arranged so that early maturing cultivars are down-wind from late maturing cultivars (Roelfs *et al.*, 1992). Avoiding excess nitrogen and frequent irrigation are helpful in controlling stem rust (Roelfs, 1985).

Separation of winter and spring wheat grown in the same area by another crop, can delay the spread between fields (Roelfs, 1985).

1.6.2. Barberry eradication

Eradication of the alternate host was started in Rouen, France, in 1660. Successful eradication programmes in Northern Europe and the North Central States of the U.S.A. are well documented. Except for Eastern Europe and the North-Western U.S.A., no areas of the world are known where alternate hosts play a role in stem rust epidemiology (Roelfs *et al.*, 1992). After the disastrous epidemic of 1916, laws against the growing of barberry (*Berberis vulgaris* L.) were passed in the important wheat-producing areas of the U.S.A. A cooperative federal and state programme on barberry eradication was started in 1918, with E.C. Stakman as its head (Roelfs, 1982). Eradication of common barberry from the wheat fields of the central Great Plains of North America was completed, for all practical purposes, by 1928. The effect of this eradication on populations of stem rust on the source of local, often early epidemics of stem rust, was eliminated. Barberry eradication increased the average useful life of resistance genes in wheat (Roelfs & Groth, 1980). Barberry eradication affected the frequency and severity of stem rust epidemics by delaying disease onset by about 10 days, reducing the initial inoculum level, decreasing the number of pathogenic races and stabilising pathogenic races (Roelfs, 1985).

1.6.3. Fungicides

Chemical control of cereal diseases is usually not desirable due to the high costs of fungicide application as well as potential environmental hazards. However, fungicides are used world-wide to maintain production levels in wheat cultivars lacking adequate levels of disease resistance (Ireta & Gilchrist, 1994). Chemicals have so far played a minor role in stem rust control (Knott, 1989).

The history of fungicides in agricultural crops was described by Kuck, Scheinpflug & Pontzen (1995), Dunne (2002) and Russell (2006). The first foliar applications were done with elemental sulphur in the early 19th century to control powdery mildew of grape vines. The dithiocarbamates were patented in 1934 by Tisdale & Williams working for DuPont and these compounds developed into one of the most important classes of broad-spectrum, protectant fungicides. Between 1945 and 1970 other major classes of chemicals were introduced. In the mid-seventies the

DMI (demethylation inhibitors) fungicide group, which contains the triazole fungicides, was introduced. The STAR or strobilurin type fungicides were introduced in 1997.

According to Hewitt (1998), chemical sprays on wheat and barley result in 87% of the total pesticide input into cereals which at the time were equivalent to $3967 million in sales. Since 1986, 16 out of 26 new fungicides have been aimed initially at the cereal sector. The leading cereal fungicides are mobile, specific compounds and are mainly triazoles or morpholines.

- **Foliar application**

The grain yield of cereals depends to a large degree on how long the leaves are able to remain green. Some fungicides have the ability to have a stay-green effect (Gordon & De Villiers, 1989). The reason for the minor role of fungicides in stem rust control can be the effectiveness of host resistance, the rate of disease increase for wheat stem rust under ideal conditions and the relatively low economic return per hectare of wheat in comparison to the cost of fungicide applications (Rowell, 1985). According to Nel *et al.* (1999) only tebuconazole (Folicur®) is registered for control of wheat stem rust in South Africa. However, ACDASA and CropLife South Africa list tebuconazole, triadimefon, propiconazole, and cyproconazole plus propiconazole, as registered wheat stem rust fungicides (ProCrop™ Professional software version 1.30). Gordon & De Villiers (1989) found that stem rust control with tebuconazole was comparatively modest, possibly because the lower parts of the stem may not have been adequately wetted, particularly in the later stages of growth when the flag leaf had appeared.

According to research done by Loughman, Jayasena & Majewski (2005), the fungicide Folicur® was more effective at reducing disease and increasing yield or quality than Impact® or Triad®. Fungicides reduced stem rust severity on plant parts that were only slightly infected at that time, but were not effective on heavily affected plants. Fungicides applied at head emergence increased yield by 0.8-1.5 $t.ha^{-1}$, depending on the control.

- **Seed treatment**

The control of rust diseases by treating seed with a systemic fungicide is an attractive option. Research has shown that compounds with extremely low dosage response against the pathogen can be used effectively in controlling rust diseases (Rowell, 1985). Seed treatments can be used in combinations with foliar sprays when cultivars are very susceptible, alone when yields are too low to justify foliar sprays, and in combination with slow rusting resistance to stem rust (Knott, 1989).

Hagborg (in Mills & Kotze, 1978) stated that the sulphone analogue, oxycarboxin or DCMO, reduced the incidence of stem rust significantly under field conditions. He also found that oxycarboxin 75% wettable powder (WP) applied at 4.2 kg/ha, controlled stem rust just as effectively as nickel compounds plus zineb. Formulations of DCMO plus thiram or carboxin/thiram have been developed for use on small grains (Mills & Kotze, 1978).

Fungicide resistance often develops in fungal populations. The use of integrated pest management (IPM) practices, disease forecasting and research inputs may reduce the frequency of resistance development (Hewitt, 1998). A committee (FRAC, Fungicide Resistance Action Committee) has been established to review each fungicide type and to determine the compounds at risk. They have classified fungicides into groups according to their mode of action. Fungicides belonging to the same category can result in resistance if that specific group is used too frequently.

Due to high costs the research into and development of new compounds, have been reduced to a few multi-national pesticide companies. The effective use of modern fungicides and thus the desired economic benefits, require a high level of education for all role players. The use of modern fungicides requires a more integrated approach and complete control of most diseases of wheat can be done by one to three applications of fungicides (Lyr, 1995).

1.6.4. Breeding for resistance

Genetic resistance is the most effective, environment-friendly and economical way to control stem rust of wheat. The use of resistant cultivars adds no extra cost to farmers because there are no chemicals to buy or additional cultural operations to be carried out (Knott, 1989). Genetic resistance occurs when a resistance allele is present in the host along with a corresponding avirulence gene in the pathogen (Johnson & Knott,

1992). Several types of resistance to stem rust in wheat, including seedling resistance, adult-plant resistance, and slow rusting, have been described.

The primary objective of a rust genetics programme is to understand the expression and inheritance of resistance and to know the range of available genetic diversity (McIntosh, 1988a). However resistance breeding is regularly confronted by genetic adaptation in the pathogen. Virulent pathotypes often increase in frequency and either render the resistant cultivars vulnerable to disease or actually cause crop losses ("boom and bust" cycles) (McIntosh, 1988a).

1.6.5. Types of resistance

- **Seedling resistance**

Seedling (qualitative) resistance, also known as complete resistance, usually protects the plant against avirulent pathogen isolates during their entire growing period. Seedling resistance is often of the pathotype-specific, major gene type (Knott, 1989). When used extensively over time and space, new pathotypes usually circumvent seedling resistance in a relatively short period after the release of such cultivars (Rajaram, Singh & Torres, 1988). The longevity of resistance based on major genes may be limited and stepwise mutation can eventually lead to susceptibility, but this strategy has been successfully employed in Australia (Rajaram *et al.,* 1988).

A range of designated and temporary designated *Sr* genes, controlling stem rust resistance, has been described (Table 3). Most of the seedling genes have become ineffective after their incorporation in cultivars in different countries, but by managing seedling resistance, the lifespan of these genes can be lengthened. The stacking of genes into a specific cultivar can provide protection for several years (Roelfs *et al.,* 1992). In addition, post-seedling resistance genes used in combination with seedling resistance should reduce the rate of build-up of a new race with virulence on seedling resistance genes (De Pauw & Buchannon, 1975).

- **Adult-plant resistance (APR)**

Plants with APR are susceptible at the seedling stage and develop resistance in post-seedling phases (Roelfs, 1985). General resistance is effective against all races of the pathogen, while specific resistance (seedling) is effective against

some races and ineffective against others (Knott, 1982). Nazareno & Roelfs (1981) described the value of APR in protecting wheat cultivars against stem rust. Efforts should be made to combine those genes in a single cultivar that confer some degree of general resistance against the pathogen. Preliminary genetic studies of a large number of selected lines that have APR, indicated that resistance was generally recessive and controlled by several genes with small effects (Knott & Padidam, 1988).

The APR gene *Sr2*, although less effective individually, proved to be a durable source of resistance in many parts of the world. There is no doubt that *Sr2* provides a desirable genetic background into which more effective, but less durable genes can be placed (McIntosh, 1988b). Stem rust resistance in wheat conferred by *Sr2* has remained effective against stem rust worldwide for more than 50 years (Spielmeyer & Lagudah, 2003). The presence of *Sr2* in South African wheat cultivars such as Palmiet was considered valuable in terms of anticipated durability of resistance to stem rust of wheat (Pretorius & Brown, 1997). According to Sunderwirth & Roelfs (1980) *Sr2* should be useful in combinations with other genes as a back-up resistance in high-risk stem rust regions.

- **Slow rusting**

Slow rusting is incomplete or quantitative resistance that is associated with a reduced rate of epidemic development (Roelfs & McVey, 1979). Slow rusting may be the result of fewer and smaller uredinia, longer latent periods and slower growing lesions resulting in less stem area infected (Martin *et al.*, 1979). The ability to retard development of wheat stem rust is apparently effective in reducing yield losses (Wilcoxson, Skovmand & Atif, 1975). Some “defeated” resistance genes expressed significant residual effects in the form of reduced infections and in sporulation capacity. By combining a number of “defeated” race-specific resistance genes non-specific or rate-reducing resistance could be obtained (Brodny, Nelson & Gregory, 1986).

Slow rusting results from limited growth in the host after penetration has occurred (Martin, Littlefield & Miller., 1977). According to Palmer & Wilcoxson (1982), infection frequency and latent period were not affected by the plant’s

anatomical and morphological characteristics, but that enlargement of uredinia and sporulation by the pathogen could be affected by plant structure.

The term ‘slow rusting’ originates from the disease phenotype and has no genetic meaning.

1.6.6. Sources of resistance

Due to the continual evolution of rust pathotypes, there must be a constant search for germplasm possessing resistance to the cereal rusts. Several natural sources of the specific type of resistance are available, namely cultivars, land races or primitive cultivars, wild or cultivated relatives and mutations (Dyck & Kerber, 1985). Numerous sources of resistance exist to rust diseases, although not all are of equal value (McIntosh *et al.*, 1995). Genes conferring resistance to stem rust were identified in *T. aestivum, T. boeoticum, T. durum, T. dicoccum, T. monococcum, T. timopheevi, T. turgidum, A. elongatum, Ae. squarrosa* and *S. cereale* (Dyck & Kerber, 1985).

When the various related species have a genome(s) that is homologous with at least one of the genomes of cultivated wheat, transfer of resistance is relatively simple (Dyck & Kerber, 1985). Bridge crosses may be used where the transfer of genetic material, usually between different levels of ploidy, is difficult or impossible by direct hybridisation (Dyck & Kerber, 1977). The use of cytogenetic procedures is available to genetically exchange chromosomes from distantly related species (Dyck & Kerber, 1985).

1.6.7. Molecular markers

Markers used in plant breeding programmes fall into three broad categories, namely morphological linked disease resistance genes, biochemical and DNA based markers. Markers should be closely linked to the gene controlling an economically important trait (Eagles *et al.*, 2001). The large scale application of markers that are available in wheat breeding are PCR based markers such as microsatellites (SSR) and STS markers developed from sequencing such RFLP, AFLP or RAPD markers (William, Trethowan & Crosby-Galvan., 2007). Some STS markers for resistance genes such as *Sr24, 25, 26* and *38* are available and are implemented in the marker-assisted selection (MAS) wheat breeding programme at CIMMYT (William *et al.*, 2007).

The use of pseudo-black chaff, or high-temperature-induced seedling chlorosis as morphological markers to detect the stem rust resistant gene *Sr2,* has been of economic importance in breeding programmes in Australia (Eagles *et al.,* 2001). Other morphological markers used in their breeding programme are red-glumes associated with *Yr10* and leaf tip necrosis (LTN) linked to *Lr34* (Eagles *et al.,* 2001). In South Africa, marker development has focused primarily on the leaf rust resistance gene *Lr19* and stripe rust resistance in cv. Kariega. The use of markers in South Africa to identify useful rust resistance genes has been successful to some extent, but their application in breeding programmes could be improved (Pretorius *et al.,* 2007).

Useful markers are likely to become available for traits of importance in the near future. However, the cost associated with MAS assays is the limiting factor for its adoption in public wheat breeding programmes. The use of markers also depends on the breeding objectives and target traits (William *et al.,* 2007). The complexity of the genome of hexaploid wheat has made it a relatively difficult species for marker application (Eagles *et al.,* 2001).

1.6.8. Durability

In breeding for resistance, the main objective is to produce cultivars with durable resistance. Plant breeders generally agree that breeding for resistance should not depend solely on race-specific genes. In a wheat breeding programme it would be desirable and in some cases essential, to incorporate a number of genes into one rust-resistant variety. Thus, resistance would be provided to more pathotypes and resistance to individual pathotypes would probably be increased (Knott & Anderson, 1956).

Long-term resistance to stem rust is dependent on a continuing availability of resistance sources. Various procedures assist in ensuring that potential resistance sources carry new or different genes for resistance (McIntosh, 1988a). By interpreting pathogenic surveys and a reasonable knowledge of the genetics of resistance, a durable source of resistance could be found (Knott, 1989). Resistance that is race non-specific and controlled by a number of genes may be long-lasting because directional selection pressure on the pathogen will be minimal (Knott & Padidam, 1988). Although theoretically non-durable, losses due to stem rust have been successfully reduced through the use of wheat cultivars possessing vertical resistance (Eaton, McVey & Busch, 1984).

Knowledge of genetic variation for virulence and of host resistance effective to these variants, is the basis for utilisation of diverse resistance in the development of wheat cultivars resistant to the pathogen. It is well known that diversity can be introduced into a crop community at a number of levels and in a number of ways. One of the methods for extending the field life of varieties, the development of "multigene and multiline" cultivars, also requires information on the effectiveness of known genes for resistance against local virulences (Sawhney & Goel, 1981).

Kolmer, Dyck & Roelfs (1991) researched stem rust resistance of wheat grown in Western Canada and found that cultivars with a combination of effective genes have been more resistant over a long period of time than closely related cultivars that have fewer of the same resistance genes. Breeding for durable resistance should be based on tests with pathotypes that enables the desired resistance genes to be selected (Lombard, 1986). This necessitates the regular surveying of wheat regions for prevailing pathotypes.

1.6.9. The effect of environment on host-pathogen interactions

Watson (1970) proposed that virulence is controlled by specific genes whose products interact with those of corresponding resistance genes in the host, under a relatively simple genetic system. Growth rate, lesion size, spore production, aggressiveness and characters related to pathogenicity are controlled by a polygenic system. Watson (1970) further suggested that survival ability was related to characters controlled by a genetically similar, but different system. In reviewing the literature Browder (1985) stated that the mechanisms controlling host specificity are influenced by the environment.

Loegering (1963) referred to the effects of environment upon both growth and expression of the host-pathogen interaction. Hart (1955) reported that both 'Kenya 58' and 'Kenya 117A' became moderately susceptible to race 15B at high temperatures. Loegering (1963) studied near-isogenic lines, with and without *Sr6*, at different temperatures and found that the lines possessing *Sr6* were resistant to an avirulent pathogen culture only at low temperatures.

Luig & Rajaram (1972) noted the interaction of higher temperatures with susceptible genetic backgrounds in decreasing the degree of resistance conferred by all the genes with which they worked. In a study on resistance controlled by *Sr6*, Knott (1981) concluded that a complex interaction involving genotype, temperature

and light exists. Knott & Anderson (1956) found that increased post-inoculation temperatures applied to seedlings with *Sr6* resulted in reduced resistance following subsequent inoculation with avirulent cultures. Silverman (1959) concluded that the development of necrosis associated with rust infection was particularly sensitive to temperature.

According to McIntosh *et al.* (1995) genes *Sr6, 8b, 10, 12, 13, 14, 15, 17, 22, 23, 34, 36,* and *38* are sensitive to environmental variation. In addition to qualitative differences in *Sr* gene phenotype, Browder (1985) concluded that quantitative variation in host response is likely to be the result of parasite-host-environment specificity.

1.7 CONCLUSIONS

Wheat is the world's most important crop and the rusts are present wherever wheat is grown. Stem rust is the most researched host-pathogen system in agriculture. However, much remains to be discovered in the areas of both applied and basic research. The occurrence of stem rust epidemics in South Africa depends largely on the ability of the fungus to over-summer on volunteer plants, environmental conditions suitable for pathogen survival and growth, and the cultivation of susceptible cultivars. Since there are no known alternate hosts for the fungus in South Africa, the pathogen changes through mutation and introduction of exotic pathotypes.

Breeding resistant cultivars is the most effective method of stem rust control. Breeding efforts in South Africa should concentrate on combining quantitative resistance and effective seedling genes (gene-pyramiding). To increase the possibility of achieving durable resistance, breeders should select parents with satisfactory agronomical traits and proven long-lasting resistance. The deployment of cultivars with APR genes and a combination of seedling genes are recommended in areas where stem rust is a major problem.

Effective genetic control of rust diseases requires a coordinated effort, including pathotype monitoring, collection and characterisation of sources of resistance and resistance breeding. More efficient traditional and molecular-based selection techniques should be maintained or developed. The continued development

of cultivars with a combination of different types of resistance can reduce inoculum and prevent the pathogen population from increasing to epidemic levels in future.

The primary objective of this study was to identify fungicides toxic to *P. graminis* f. sp. *tritici.* A chapter on pathogenic variability of wheat stem rust is also included.

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Table 1. Virulence surveys of *Puccinia graminis* f. sp. *tritici* that is generally available in international literature.

Country	**Year**	**Reference***
Australia	1990-91	Park (1991)
Brazil	1982-85	Roelfs *et al.* (1992)
Bulgaria	1974-78	Roelfs *et al.* (1992)
Canada	1992	Harder *et al.* (1994)
Czechoslovakia	1981-83	Roelfs *et al.* (1992)
Egypt	1974-76	Roelfs *et al.* (1992)
Ethiopia	1982-83	Roelfs *et al.* (1992)
France	1977	Roelfs *et al.* (1992)
Germany	1965-66	Roelfs *et al.* (1992)
Greece	1955-62	Skorda (1966)
Hungary	1969-72	Roelfs *et al.* (1992)
India	1985	Bahadur (1985)
Iraq	1967-69	Roelfs *et al.* (1992)
Italy	1984	Roelfs *et al.* (1992)
Kenya	1969-70	Roelfs *et al.* (1992)
Korea	1971-72	Roelfs *et al.* (1992)
Mexico	1982	Singh (1991)
Pakistan	1976	Roelfs *et al.* (1992)
Portugal	1980	Roelfs *et al.* (1992)
Romania	1968-70	Roelfs *et al.* (1992)
South Africa	1991-97	Boshoff *et al.*, 2000
Spain	1968-71	Roelfs *et al.* (1992)
USA	1994	McVey *et al.* (1996)
USSR	1971	Roelfs *et al.* (1992)
Uruguay	1968	Roelfs *et al.* (1992)
Yugoslavia	1976-83	Roelfs *et al.* (1992)

* Updated references

Table 2. Avirulence/virulence formulae for stem rust pathotypes in South Africa up to the end of 2000.

Pathotype	Avirulence/virulence formula (*Sr*-genes)
2SA2	*Sr5,6,8b,9b,9e,17,24,27,30,36,38/Sr7b,9g**
2SA4	*Sr8b,9g,24,27,36,38/Sr5,6,7b,9b,9e,11,17,30**
2SA6	*Sr8b,9e,24,27,36,38/Sr5,6,7b,9b,9g,17,30**
2SA10	*Sr6,7b,8b,9b,9e,17,24,27,30,36,38/Sr5,9g**
2SA18	*Sr7b,8b,9b,9e,24,27,30,36,38/Sr5,6,9g,17**
2SA20	*Sr8b,9e,24,27,30,38/Sr5,6,7b,9b,9g,17,36**
2SA32	*Sr5,6,7b,8b,9b,9e,9g,17,24,30,36,38/Sr11**
2SA33	*Sr7b,8b,9e,9g,17,24,27,30,38/Sr5,6,9b,36**
2SA36	*Sr7b,8b,9e,9g,24,27,30,38/Sr5,6,9b,11,17,36**
2SA39	*Sr5,8b,9b,9e,17,24,27,30,36,38/Sr6,7b,9g**
2SA43	*Sr8b,24,27,36,38/Sr5,6,7b,9b,9e,9g,17,30**
2SA45	*Sr8b,9e,9g,24,27,36,38/Sr5,6,7b,9b,17,30**
2SA48	S*r8b,9e,9g,24,27,30,36,38/Sr5,6,7b,9b,17**
2SA49	*Sr8b,9e,9g,24,27,38/Sr5,6,7b,9b,17,30,36**
2SA51	*Sr8b,9b,9e,9g,17,24,27,30,36,38/Sr5,6,7b *
2SA52	*Sr8b,9e,24,27,30,36,38/Sr5,6,7b,9b,9g,17**
2SA53	*Sr8b,24,27,38/Sr5,6,7b,9b,9e,9g,17,30,36**
2SA54	*Sr7b,8b,9e,9g,24,27,36,38/Sr5,6,9b,17,30**
2SA55	*Sr11/Sr5,6,7b,8b,9b,9e,9g,17,24,27,30,36,38***
2SA88	*Sr24,27,36/Sr5,6,7b,8b,9b,9e,9g,17,30,38***
2SA100	*Sr7b,8b,9e,9g,27,30,36,38/Sr5,6,9b,17,24**
2SA101	*Sr8b,9e,9g,30,36,38/Sr5,6,7b,9b,9g,17,24**
2SA102	*Sr5,6,7b,8b,9b,9e,11,17,24,36,38/Sr9g,27**
2SA102(K)	*Sr5,6,7b,8b,9b,9e,11,17,24,36,38/Sr9g,27,30,*(Kiewiet)****
2SA103	*Sr5,6,7b,8b,9b,9e,9g,11,17,24,36,38/Sr27,30**

* Le Roux & Rijkenberg, 1987b

****** Boshoff *et al.*, 2002

******* Unpublished data

Table 3. Designated and temporarily designated resistance genes for *Puccinia graminis* f. sp. t*ritici* (http://www.ars.usda.gov)

Sr gene	Genome location	Linkage	Original source	Tester line	Remarks
Designated					
1				see *Sr9d*	
2	3BS		*Triticum turgidum* (Yaroslav emmer)	CnS(Hope3B)	Few uredinia APR
5	6DS		Reliance	I*Sr5*-Ra	
6	2DS	*Lr2, L 15*	Red Egyptian	I*Sr6*-Ra	Test at 18°C
7a	4BL		Kenya 117 A	Line G Sel	
7b	4BL		Marquis	I*Sr7b*-Ra	
8a	6AS		Red Egyptian	I*Sr8*-Ra	
8b	6AS		Barletta Benvenuto	Barletta Benvenuto	
9a	2BL		Red Egyptian	I*Sr9a*-Ra	
9b	2BL		Kenya 117 A	W2691 *Sr9b*	
9d	2BL		Hope	I*Sr9d*-Ra	
9e	2BL		*Triticum turgidum* (Vernal emmer)	Vernstein	
9f	2BL		Chinese Spring	Chinese Spring	
9g	2BL	*Yr7*	Lee	CnS*Sr9g*	

Table 3 (cont.). Designated and temporarily designated resistance genes for *Puccinia graminis* f. sp. t*ritici* (http://www.ars.usda.gov)

Sr gene	Genome location	Linkage	Original source	Tester line	Remarks
Designated					
10	2B		Egypt NA95	W2691 *Sr10*	
11	6BL		Lee	I*Sr11*-Ra	
12	3BS		Thatcher	Bt*Sr12*Tc	Test at 18°C
13	6AL		*Triticum turgidum* (Kaphli emmer)	W2691 *Sr13*	Test at 25°C
14	1BL		*Triticum turgidum* (Kaphli emmer)	Line A Sel	
15	7AL		Norka	W2691 *Sr15*	Test at 18°C
16	2BL		Thatcher	I*Sr16*-Ra	
17	7BL		*Triticum turgidum* (Yaroslav emmer)	CS (Hope7B)	Test at 18°C
18	1D		Marquis	Lc*Sr18*Mq	
19	2BS		Marquis	Lc*Sr19*Mq	
20	2BL		Marquis	Lc*Sr20*Mq	
21	2AL		*T. monococcum*	Einkorn	
22	7AL		*T. monococcum*	Sw*Sr22* T.B.	

Table 3 (cont.). Designated and temporarily designated resistance genes for *Puccinia graminis* f. sp. *tritici* (http://www.ars.usda.gov)

Sr gene	Genome location	Linkage	Original source	Tester line	Remarks
Designated					
23	2BS	*Lr16*	Exchange	Exchange	
24	3DL	*Lr24*	*A. elongatum*	Bt*Sr24*Agt	
25	7DL	*Lr19*	*A. elongatum*	Lc*Sr25*Ars	
26	6AL		*A. elongatum*	Eagle	
27	3A		*Secalis cereale* (Imperial)	W2691 *Sr27*	
28	2BL		Kota	W2691 *Sr28*Kt	
29	6DL		Etiole de Choisy	Pusa	
30	5DL	*Pm2*	Webster	Bt*Sr30*Wst	
31	1BL	*Lr26, Yr9*	*S. cereale* (Imperial)	Kavkaz	
32	2A, 2B		*T. speltoides*	ER 5155	
33	1DL	*Lr21*	*T. tauschii*	RL 5288	
34	2A, 2B	*Yr8*	*T. comosa*	Compare	
35	3AL		*T. monococcum*	Mq(2)5xG2919	
36	2BS		*T. timopheevi*	W2691 *SrTt-1*	
37	4AL		*T. timopheevi*	W2691 *SrTt-2*	Off type plants

Table 3 (cont.). Designated and temporarily designated resistance genes for *Puccinia graminis* f. sp. t*ritici* (http://www.ars.usda.gov)

Sr gene	Genome location	Linkage	Original source	Tester line	Remarks
Designated					
38	2AS	*Lr37, Yr17*	*T. ventricosa*	VPM1	Test at 18°C)
39	2B	*Lr35*	*T. speltoides*	RL 5711	
40	2BS	*Lr13,23,16*	*T. araraticum*	RL 6087	
41	4D		Waldron	Waldron	
42	6D		Norin 10	Norin 10	
43	7D		*A. elongatum*	KS10-2	
44	7DS		*A. intermedium*	Taf-2	
45	1DS		*T. tauschii*	RL 5289	
Temporary designated					
Bj			Bejon	Bejon	
Charter			Charter	Charter	
Dp-2	6AS		Golden Ball	Media	
Em			Entrelargo de Montijo		
Gt			Gamut	Gamut	
H			H-44	H-44	

Table 3 (cont.). Designated and temporarily designated resistance genes for *Puccinia graminis* f. sp. t*ritici* (http://www.ars.usda.gov)

Sr gene	Genome location	Linkage	Original source	Tester line	Remarks
Temporary designated					
Kt2	2BL		Kota	Line AE Sel	
LC			Little Club	Little Club	
McN			McNair 701	McNair 701	
Satu			Satu triticale	Satu	
Tmp	4B		Truimph 64	Truimph	
Tt-3			*T. timopheevi*	Fed*2/*SrTt-3*	
U	2D		Red Egyptian	CnsSrUre	
Wld1			Waldron	Bt*SrWld*	
Wld2			Webster	Webster	
Zdar	1B			Zdar	
A	2D		Coteau	Coteau	
B	2BL		Coteau	Coteau	
C	2B		Len	Len	

Figure 1. Orange-red pustules of *Puccinia graminis* f. sp. *tritici* on the stem of a susceptible plant (image courtesy of Z.A. Pretorius).

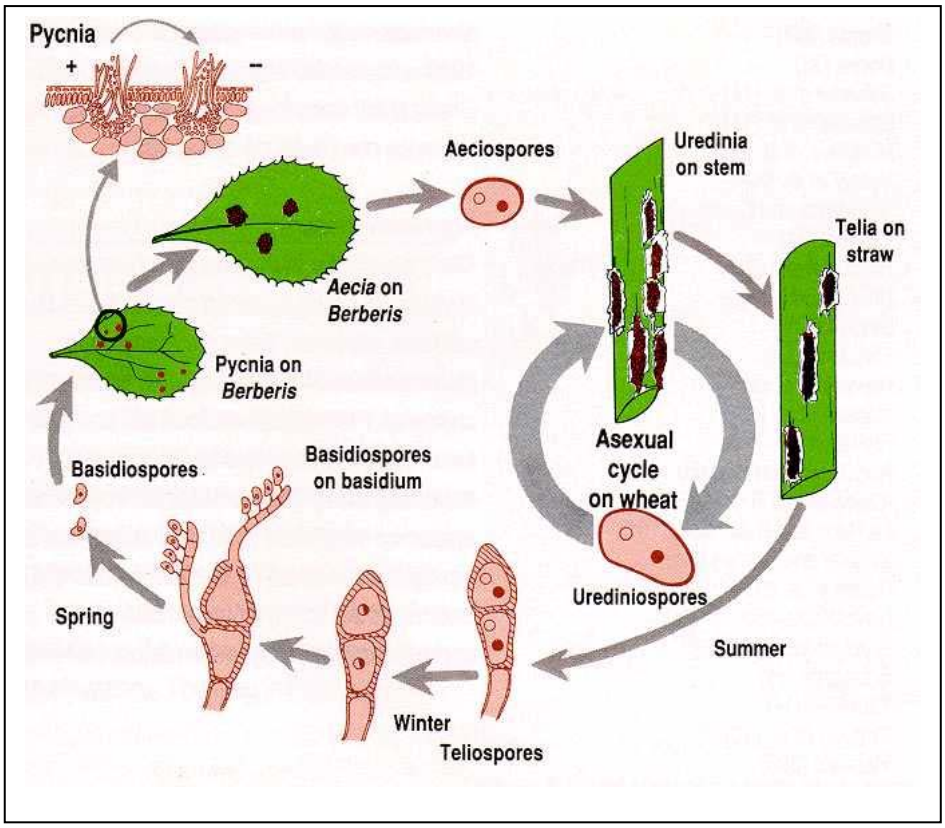

Figure 2. The life cycle of *Puccinia graminis* f. sp. *tritici* as noted in the northern hemisphere (Roelfs *et al.*, 1992).

CHAPTER 2

PATHOTYPES OF *PUCCINIA GRAMINIS* F. SP. *TRITICI* DETECTED IN SOUTH AFRICA FROM 1998-2004

2.1 ABSTRACT

A stem rust survey is conducted annually to monitor pathogenic variability in *Puccinia graminis* f. sp. *tritici* in the major bread wheat and triticale (X *Triticosecale* Wittmack) producing areas of South Africa. Data generated from pathotype surveys form an essential component of breeding programmes. Collections from trap nurseries and commercial wheat fields were multiplied on McNair 701 seedlings in a glasshouse. Single uredinium-derived isolates were inoculated onto a set of differential wheat lines and pathotypes determined according to the avirulence and virulence profile of each isolate. During 1998 to 1999 the incidence of stem rust on susceptible wheat and triticale cultivars and lines planted in trap nurseries was low. Four pathotypes were detected from 1998 to 1999, with 2SA4 (43%) and 2SA102 (45%) being dominant. A new pathotype, 2SA99, was found in 1999 and, in 2003, a new pathotype similar to 2SA102, but virulent to Kiewiet triticale (2SA102K), was detected. During the 2000 season, 40 single pustule isolates were established. Two new pathotypes were identified, namely 2SA88 and 2SA55. Pathotype 2SA55 was most frequently isolated, constituting 65% of all collections. In 2001 16 isolates were evaluated of which 2SA88 constituted 68%. Sixty two, 114 and 122 stem rust collections were analyzed in 2002, 2003 and in 2004, respectively. More than 70% of the isolates obtained from 2002 to 2004 were characterized as pathotype 2SA88, which unusually was virulent for *Sr8b* and *Sr38*. During the six year survey period, stem rust pathotypes 2SA4, 2SA55, 2SA88, 2SA99 and 2SA100 were detected on bread wheat and 2SA102, 2SA102K and 2SA103 on triticale. The steady increase in stem rust occurrence appears to be directly related to the market share of susceptible cultivars and continued breeding for durable resistance is required. Finally some suggestions are made to improve the stem rust differentiating set in South Africa.

2.2 INTRODUCTION

Stem rust caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*) is an economically important disease of bread wheat (*Triticum aestivum* L.) worldwide (Roelfs, Singh & Saari, 1992; McIntosh, Wellings & Park, 1995). Variation in severity of annual rust epidemics is influenced by the rust species, environmental factors, cultural practices and cultivar resistance (Eversmeyer & Kramer, 2000).

In South Africa wheat and barley (*Hordeum vulgare* L.) were first produced by Dutch settlers at the Cape of Good Hope during 1652 (Lombard, 1986). Currently bread wheat is produced in three production systems in South Africa, namely rain-fed spring wheat (Western and Southern Cape), irrigated wheat (Northern Cape, Northwest and KwaZulu-Natal) and rain-fed winter wheat (Free State). Stem rust infections have historically occurred in most production regions of South Africa but have more recently been restricted to spring wheat in the Western Cape. Stem rust has often reached epidemic proportions in South Africa (Lombard, 1986), with the most recent being severe and widespread infection of *Sr24*-derived wheat cultivars in 1985 (Le Roux, 1985; Le Roux & Rijkenberg, 1987a,b). However, due to efficient resistance breeding levels of stem rust in wheat crops, and the diversity of *Pgt* pathotypes recovered in surveys, have been lower during the past two decades (Boshoff, Van Niekerk & Pretorius, 2000).

The timely detection of stem rust pathotypes with new virulence is considered important to the South African wheat industry and a shift in virulence that can contribute to cultivar susceptibility, and thus potential losses, is important to producers aiming for maximum yields. The first pathotyping of stem rust in South Africa was initiated by Verwoerd in the 1920s (Verwoerd, 1931; 1935). Although annual surveys were not conducted for most of the 20th century, available information on pathogenic variability was summarized by Lombard (1986). In South Africa, 22 pathotypes of *Pgt* that occur on wheat and triticale have been characterised between 1981 and 1997 (Le Roux & Rijkenberg, 1987a; Le Roux, 1989; Smith & Le Roux, 1992; Boshoff *et al*., 2000).

The objectives of the surveys were to (a) determine the occurrence and distribution of stem rust in South Africa; (b) timeously detect new pathotypes originating either by means of mutation or exotic introduction; (c) determine the

avirulence/virulence reactions for each pathotype; (d) maintain an appropriate pathotype collection to test cultivars and breeders' lines, and (e) enhance the knowledge of the pathogen and its distribution from year to year.

In this chapter the results of pathogenicity surveys are presented for wheat, barley and triticale stem rust collections made during 1998-2004.

2.3 MATERIALS AND METHODS

Wheat fields and disease nurseries were surveyed periodically from 1998 - 2004 by researchers of the ARC-Small Grain Institute (SGI), Bethlehem, South Africa, to monitor the occurrence, development and distribution of stem rust in South Africa. Disease nurseries, planted annually in the most important wheat production areas of South Africa, were visited at least once each wheat season.

Disease nurseries included two universally susceptible cultivars McNair (stem and stripe rust susceptible) and Morocco (leaf, stem, and stripe rust susceptible), a stripe rust differential set, several tester lines carrying different stem and leaf rust resistance genes, a number of commercial wheat cultivars and advanced breeding lines. During surveys, sections of bread wheat, barley and triticale stems infected by stem rust, and exhibiting sporulating uredinia, were placed in glassine bags and transported to the SGI. In addition, rust samples were sent to the SGI by co-workers responsible for the planting and maintaining of disease nurseries. Details were recorded of the date of collection, location, cultivar if known and rust severity.

On receipt of samples, urediniospores were harvested using a cyclone collector. The collected spores, suspended in a light-weight mineral oil (Soltrol® 170), were applied to primary leaves of wheat cv. McNair 701 seedlings which had previously been treated with maleic hydrazide (50 mℓ/pot of 0.3 gℓ^{-1}) to enhance urediniospore production and to retard plant growth. Inoculated seedlings were placed in an incubation chamber, held at >96% relative humidity for a 14 h dark period, followed by a 3 h gradual drying period at a light intensity of 120 µE/m^2/s. Temperature inside the chamber was maintained at 20 ±2°C. Plants were then moved to a greenhouse where natural daylight were supplemented with illumination from cool-white fluorescent tubes for 16 h each day and where temperature was controlled

at 22±2°C. After 10 days, urediniospores were collected from a single uredinium and increased on 7-d-old McNair 701 seedlings to produce sufficient spores to inoculate a differential set.

The differential set consisted of Reliance (*Sr5*), McMurachy (*Sr6*), Marquis (*Sr7b*), ISr8A-Ra (*Sr8a*), Barleta Benvenuto (*Sr8b*), W2402 (S*r9b*), Vernal (*Sr9e*), Acme (*Sr9g*), Yalta (*Sr11*), Renown (*Sr17*), Einkorn (*Sr21*), Gamka (*Sr24*), Coorong (*Sr27*), Festiguay (*Sr30*), Gamtoos (*Sr31*), W2691 (*Sr36*), VPM1 (*Sr38*), Taf-2 (*Sr44*) and McNair 701 (susceptible control). Inoculated sets were exposed to the same incubation procedures as described above. Stem rust pathotypes were identified according to their avirulence/virulence profiles.

To confirm host responses to different *Pgt* pathotypes and compare differential sets, isolates representative of 2SA4, 2SA36, 2SA88, 2SA55, 2SA100, 2SA102, 2SA102K and 2SA103 were inoculated onto the South African and North American stem rust differential sets. Trident (*Sr38*), Kiewiet and Tobie (*SrSatu*), both triticales, were added to the South African set. Seed of the North American differentials were obtained from the ARS-USDA Cereal Disease Laboratory (CDL), St Paul, Minnesota. Original CDL stocks, without a cycle of seed multiplication, were used. These tests, which were conducted at the University of the Free State under conditions similar to those described above, allowed allocation of North American *Pgt* race codes.

2.4 RESULTS

During 1998-2004, 395 single stem rust pustules were obtained from stem rust samples collected from wheat and triticale cultivars and lines throughout the small grain production areas of South Africa. Of the 395 isolates, 60% originated from the Southern Cape, 28% from the Western Cape, 6.3% from Mpumalanga, 3.1% from KwaZulu-Natal, 1.5% from Free State and 1.1% from Lesotho (Table 1). Eight stem rust pathotypes were detected, namely 2SA4, 2SA55, 2SA88, 2SA99 and 2SA100 from wheat, and 2SA102, 2SA102K and 2SA103 from triticale. The avirulence/virulence combination of each pathotype is presented in Table 3.

- **1998 to 1999**

During 1998 to 1999 the incidence of stem rust was low. Fungicides were regularly used on the commercial fields throughout the season to control *P striiformis* f. sp. *tritici*. Two pathotypes were detected during the 1998 season, namely 2SA4 (66%) and 2SA103 (33%) out of six samples. A new pathotype, 2SA99, was identified during the 1999 season. A total of 36 samples were collected and pathotypes 2SA4 (39%), 2SA99(5%), 2SA102 (53%) and 2SA103 (3%) were recorded.

- **2000**

No incidences of stem rust in commercial fields were found, most probably due to below normal rainfall during the production season. Samples were collected only from trap nurseries in the Southern and Western Cape and 40 single pustule isolates were established. Two new pathotypes were identified, namely 2SA88, unusually virulent to *Sr8b* and *38*, and 2SA55, virulent to *Sr11* and *44*. Pathotype 2SA55 was most frequently isolated, constituting 65% of all collections and 2SA88 (35%).

- **2001**

The Swartland area of the Western Cape experienced a wetter period from June to October and the climatic conditions were favourable for stem rust throughout the season. Fungicides were frequently applied to control Septoria diseases and in some instances eyespot. The Rûens area had little rainfall and crop losses were experienced due to drought stress. Only 16 samples were collected from these regions, yielding pathotypes 2SA88 (68%), 2SA99 (18%) and 2SA102 (12%).

- **2002**

Stem rust was first found in the Sandveld area of the Western Cape on the cultivar SST88 in September 2002. It was the first sample collected in commercial fields since the late eighties and only isolated pustules were found. Environmental conditions were favourable for stem rust in the Western Cape but in the southern Cape it was again a dry year. During the season, 62 samples were obtained from which pathotypes 2SA88, 2SA99, 2SA102 and 2SA103 were described. Pathotype 2SA88 consisted of 81% of the samples collected during this season.

- **2003**

Weather records for the Swartland indicated the lowest rainfall in decades, while the rainfall in the Rûens was on average 140 mm more than the Swartland. In total 114 isolates of *Pgt* were processed from 57 samples collected during September to December. Most samples originated from the Western Cape. The most common pathotype was 2SA88, representing 85% of the pathotypes found. A new pathotype on the triticale cultivar Kiewiet was detected in the southern Cape near Caledon. Based on the differentials used its avirulence/virulence combination was the same as for pathotype 2SA102, therefore the new pathotype was described as 2SA102K.

- **2004**

One hundred and twenty two isolations were made from 64 field collections. Eight pathotypes were characterized. The Western Cape yielded pathotypes 2SA88 and 2SA102, the southern Cape 2SA36, 2SA55, 2SA88, 2SA99, 2SA100 and 2SA102 and in Mpumalanga pathotypes 2SA4, 2SA88, 2SA102 and 2SA103 were detected. During the summer, isolates were collected in the highlands of Lesotho and pathotypes 2SA88 and 2SA102 were identified. The predominant pathotype was 2SA88, consisting of 70% of isolates tested.

2.4.1. Comparison of differential sets

The experiment in which the South African and North American differentiating sets were compared produced high quality infection types (Table 2, Figure 1). Except for subtle differences in stem rust phenotype, entries containing the same resistance gene mostly displayed similar differentiating abilities. Different responses were, however, obtained for the *Sr9b* and *Sr21* tester lines. W2402-*Sr9b*, the South African differential, showed low infection types to pathotypes 2SA55 and 2SA102K. Line W2691-*Sr9b*, the North American entry, was susceptible to these two pathotypes (Figure 2). Based on the reaction of Einkorn, the original *T. monococcum* accession containing *Sr21*, no South African *Pgt* isolate had virulence for this gene. In contrast, pathotypes 2SA102 and 2SA103 produced high infection types on the North American tester Cns *T. mono* deriv. (*Sr21*) (Figure 3). The North American race

codes (Roelfs, Long & Roberts, 1993) for the pathotypes retested at UFS are given in Table 2.

In addition to the above comparison, published data (Le Roux & Rijkenberg, 1987a,b; Le Roux, 1989; Boshoff *et al.*, 2000) and unpublished ARC data were tabulated (Table 3). Infection types 0 to 2 were considered to indicate resistance (R) and 3 to 4 susceptibility (S). According to this comparison discrepancies occurred for classifying avirulence or virulence to *Sr7b, 8a, 9a, 9b, 9d, 10, 11, 17, 21, 30* and *Wld-1*. The variation was observed mostly for 2SA36, 2SA100 and the triticale pathotypes 2SA102, 2SA102K and 2SA103 (Table 2).

2.5 DISCUSSION

In South Africa ideal conditions for stem rust incidence normally prevail in the Western Cape, especially the southern Cape coastal regions. In exceptional seasons stem rust occurs in other wheat production areas, but severe epidemics have been absent in the summer rainfall regions for many years. Similar to other countries, pathogenicity of the South African *P. graminis* f. sp. *tritici* populations indicate a considerable degree of variation. Such variability in rust pathogens is influenced by migration, mutation, recombination, selection and chance (McIntosh, 1992).

During 1991-1996 no new pathotypes of stem rust were found. The low incidence of the disease can be attributed to resistance of the cultivar Palmiet (*Sr2+Sr24*) which comprised approximately 60% of the wheat cultivated in the Western Cape (Boshoff *et al.*, 2000). The absence of rust in the southern parts of South Africa subsequently reduced inoculum movement to the interior of the country. During September and October, when the southern spring wheat crop is maturing, weather systems moving in a north-easterly direction commonly occur and it is assumed that spores are transported by these frontal systems to the summer rainfall regions.

The years 1996 – 1999 produced only one pathotype namely 2SA99 (Boshoff *et al.*, 2000). In 1996 the outbreak of *P. striiformis* f. sp. *tritici* resulted in a high incidence of stripe rust on the cultivar Palmiet. Stem rust prevalence was low partly because of the new cultivar SST57 (resistant to stem and stripe rust) and the widespread use of fungicides to control stripe rust. At that time the most dominant

pathotypes found in the area were 2SA4 and 2SA102. Boshoff *et al*. (2000) mentioned that the planting of susceptible triticale in the summer and winter rainfall areas resulted in an increase of pathotype 2SA102. The stripe rust epidemics seriously challenged wheat breeding and considerable time and effort were put into controlling this disease. Consequently stem rust resistance was overlooked in most breeding programmes. Based on survey data no or little stem rust was found in commercial fields in the 1990s. Since 2002 almost every commercial field in the Western and southern Cape had a substantial amount of stem rust infections. The increase in stem rust inoculum, and subsequent *Pgt* collections made by the ARC (Figure 4), can by attributed to the release of susceptible cultivars from 2000 onwards.

Pathotypes 2SA88 and 2SA55 found in 2000 differed from those previously identified in South Africa. According to Boshoff *et al.* (2002), 2SA88 was the first local isolate to have virulence towards *Sr8b* and the *T. ventricosum*-derived gene *Sr38.* Since these virulences were uncommon in South Africa, it seems unlikely that 2SA88 was a mutant of existing pathotypes. Pretorius *et al*. (2007) mentioned that 2SA88 was similar to race Ug99 except for avirulence to *Sr31*. It is thus possible that 2SA88 originated from Eastern Africa and entered South Africa as an exotic introduction. Seedling and field reactions recorded for the barley cultivars Sterling, SSG532, Puma and Jaguar, showed an increase in susceptibility to 2SA55. The previously resistant cultivars SST57 (heterogeneous), Tugela, Tugela DN and PAN 3377 were susceptible in the seedling stage to pathotype 2SA88 (Boshoff *et al.*, 2002).

According to Boshoff *et al.* (2000) 2SA102 was expected to decline because of the release of the triticale cultivars Falcon, Korhaan, Arend and Kiewiet that were resistant to this pathotype. In 2003 an apparent single-step mutation in 2SA102 occurred and Kiewiet became susceptible. In seedling tests done at the Small Grain Institute, none of the other triticale cultivars or *Sr* genes was affected by this mutation. Based on recent infection studies, the original 2SA102 pathotype appeared avirulent to W2691-*Sr9b*. However, 2SA102K was virulent on W2691-*Sr9b*, suggesting that this pathotype mutated for virulence to both *Sr9b* and the unidentified gene in Kiewiet (Table 2). The authenticity of W2691-*Sr9b*, obtained from the Cereal Disease Laboratory in St Paul, must be considered correct (Y. Jin, ARS-USDA, personal communication). It is proposed that this line replaces W2402-*Sr9b*, which also contains *Sr7b* (Park, 2007).

The comparison of data sources suggested that South African rust pathologists should redefine its differential set and decide on entries that clearly and consistently distinguish between stem rust pathotypes. The phenotype of some genes giving variable results, e.g. *Sr7b*, *Sr17* and *Sr30*, falls in the intermediate category and misclassification is easy if appropriate control reactions are absent. Furthermore, pathotypes such as 2SA36 and 2SA100 are inherently not as aggressive as others and seldomly produce typical '4' infection types, even on susceptible hosts. What appears to be a 2 to 2++ infection type may in fact represent virulence. When compiling a new differential set, the seed should come from accredited sources. Although the North American set will be an obvious choice, present data showed that some of their entries can be replaced with other genotypes. For example, it is suggested that Combination VII be replaced with Renown as a tester for *Sr17*. The Bt/Wld (*SrWld-1*) and Cn*Sr9g* lines were heterogeneous and should be reselected for purity or replaced. It may also be meaningful to retain the North American Cns *T. mono* deriv. as a source of *Sr21* rather than Einkorn. Since Einkorn is not a high-yielding bread wheat, it is difficult to maintain sufficient seed sources and this accession appears to contain a gene in addition to *Sr21*.

Pathotypes 2SA2, 2SA6, 2SA32, 2SA43 2SA45, 2SA48, 2SA101 were last found in 1987 (Le Roux & Rijkenberg, 1987a,b; Le Roux, 1989; Boshoff *et al.*, 2000). Data presented in surveys done from 1991-1997 showed that pathotypes 2SA4, 2SA36, 2SA100 and 2SA102 prevailed during this time (Boshoff *et al.*, 2000). During the current survey period it became evident that pathotype 2SA88 dominated the stem rust populations and was found on several occasions on the cultivar SST88 in commercial fields. With the exception of the new pathotypes 2SA55, 2SA88, 2SA99 and 2SA102K, it is not clear if the others survived independently or whether they originated from inoculated breeders' plots. The fact that few stem rust samples were initially collected from commercial fields, seems to indicate that pathotypes such as 2SA4, 2SA36, 2SA100 and 2SA102 may have spread from experimental plots.

According to research done at the Small Grain Institute, the high susceptibility on cultivars tested under field conditions during the 2003 survey showed that stem rust resistance is lacking. Forty two percent of the dryland spring cultivars and 86% of irrigation cultivars tested were susceptible to stem rust in the field. High levels of susceptibility in the seedling and adult plant stages were expressed by the dryland spring cultivars SST88, SST015 and SST027 as well as by the irrigation cultivars

Kariega, Olifants, Krokodil, CRN826, SST 806, SST 822 and SST876 (SGI, unpublished data). Winter wheats were only tested as seedlings and, as a group, appeared more resistance to stem rust than the spring types.

The use of race–specific resistance to control wheat stem rust requires continued monitoring of the variation in the pathogen population for virulence (Dyck & Kerber, 1985). Long-term resistance to stem rust is dependent on a continuing availability of resistance sources. It is also unlikely that durable resistance will be achieved using single *Sr* genes. Breeders should access new germplasm on a continual basis and establish if potential resistance sources carry new or different genes for resistance (McIntosh, 1988). Worldwide virulence for *Sr2, 13, 22, 24, 26, 27, 32, 33, 34, 35, 36, 37, 39, 40, 44, Tmp* and *Tt-3* is limited (Jin *et al.*, 2007). In South Africa even fewer genes are effective against the local pathotypes.

In 1980 the Department of Agriculture decided to make stem rust resistance mandatory for wheat. Unfortunately this decision has not been upheld in recent releases of commercial cultivars. The higher incidence of stem rust in commercial fields and experimental plots can be ascribed to the growing of susceptible cultivars in the Western Cape production areas. The detection of new stem rust pathotypes is of concern to the local small grain industry and requires continued research of and breeding for durable rust resistance.

2.6 ACKNOWLEDGEMENTS

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Table 1. Frequency of pathotypes of *Puccinia graminis* f. sp. *tritici* detected in South Africa during 1998-2004.

	Pathotypes						
Year and region	**2SA4**	**2SA55**	**2SA88**	**2SA99**	**2SA100**	**2SA102**	**2SA103**
1998							
Southern Cape	4	0	0	0	0	0	2
%	66.6	0	0	0	0	0	33.3
1999							
Western Cape	0	0	0	2	0	4	0
Southern Cape	10	0	0	0	0	15	1
Mpumalanga	4	0	0	0	0	0	0
%	39	0	0	5	0	53	3
2000							
Western Cape	0	4	2	0	0	0	0
Southern Cape	0	22	12	0	0	0	0
%	0	65	35	0	0	0	0
2001							
Western Cape	0	0	8	0	0	2	0
Southern Cape	0	0	3	3	0	0	0
%	0	0	68.75	18.75	0	12.5	0
2002							
Western Cape	0	0	30	2	0	3	1
Southern Cape	0	0	2	0	0	3	3
Free State	0	0	6	0	0	0	0
Mpumalanga	0	0	12	0	0	0	0
%	0	0	81	3	0	10	6
2003*							
Western Cape	0	0	30	0	0	7	0
Southern Cape	1	0	56	1	1	2	0
Mpumalanga	0	0	2	0	0	0	0
KwaZulu-Natal	0	0	8	0	0	4	0
%	1	0	85	1	1	12	0
2004							
Western Cape	0	0	12	0	0	4	0
Southern Cape	0	4	71	3	1	17	0
Mpumalanga	1	0	1	0	0	5	1
Lesotho	0	0	1	0	0	3	0
%	0.8	3.3	69.7	0.8	0.8	23.7	0.8

*****During the 2003 survey 2 samples collected from the Southern Cape contributed to a new pathotype nl 2SA102K.

Table 2. Differentiation of *Puccinia graminis* f. sp. *tritici* pathotypes.

Tester line	***Sr* gene**	**Source**	**Pathotype[a]**							
			2SA4 PSKS	**2SA36 MJQS MJRS**	**2SA55 BNGQ**	**2SA88 PTKS TTKS**	**2SA100 PJBS PJCS**	**2SA102 GFBS**	**2SA102K BFGS**	**2SA103 GDBS**
Reliance	*5*	BV 2005	3+	3	0;	4	3	0;	0;	0
I *Sr5* Ra	*5*	CDL 2006	3++	3	0;	3++	3	0	0;	0
I *Sr6* Ra	*6*	CDL 2006	3+	3	;1	4	3	;1	;1	;1
Marquis	*7b*	BV 2005	4	3+	2	3++	2++	2+	2+	2,3
I *Sr7b* Ra	*7b*	CDL 2006	3++	3	2	3+	2+	2++	2	3
I *Sr8a* Ra	*8a*	BV 2005	3+	2+	4	3++	3	4	4	4
I *Sr8a* Ra	*8a*	CDL 2006	4	2+	3++	3++	3	3++	3+	3+
Barletta Benvenuto	*8b*	BV 1999	;1	;1	;1	4	;	;	;1	;1
I *Sr9a* Ra	*9a*	CDL 2006	4	2+	4	4	3	4	4	4
W2042	*9b*	BV 2005	3+	2++	2-	3+	2+	2	2	2
W2691 *Sr9b*	*9b*	CDL 2006	3+	2++	3	3+	2+	2	3	2++
I *Sr9d* Ra	*9d*	CDL 2006	4	3	4	3+	3	4	4	3++
Vernal	*9e*	BV 2005	3++	0;	;1	4	;	;1	1+	;1
Vernstein	*9e*	CDL 2006	4	;1	1	4	;1	1	1	1
Acme	*9g*	BV 2005	1	;1	2+	4	1	4	4	2

Tester line	***Sr* gene**	**Source**	**Pathotype[a]**							
			2SA4 PSKS	**2SA36 MJQS MJRS**	**2SA55 BNGQ**	**2SA88 PTKS TTKS**	**2SA100 PJBS PJCS**	**2SA102 GFBS**	**2SA102K BFGS**	**2SA103 GDBS**
Cn*Sr9g*	*9g*	CDL 2006	1,3	1	2+	3++	1+	4	4	2
W2691 *Sr10*	*10*	CDL 2006	3++	2++	1	3++	3	3+	4	4
Yalta	*11*	BV 2005	4	;1	3++	4	3	2	2	1+
I *Sr11* Ra	*11*	CDL 2006	3++	;1	3++	4	2-	1+	1+	1
Renown	*17*	GH 1999	3+	2	X	4	2++	;1	;1	0;
Combination VII	*13,17*	CDL 2006	2+	1	;1	2+	1-	;	;1=	;1
Einkorn	*21*	GH 1999	1	;1=	1	1	;1	1	1	1
Cns *T. mono* deriv	*21*	CDL 2006	2+	2	2	2+	1-	3	2+	2+3
Gamka	*24*	GH 2005	1	;1	;1	1	3	;	;	;
Lc*Sr24*Ag	*24*	CDL 2006	1	1	1	2	3	1	1	1
Coorong	*27*	GH 2004	;1	;	1-	;	;1	4	4	4
Festiquay	*30*	GH 1999	3+	1	2	4	2=	2	2-	2+
Bt*Sr30*Wst	*30*	CDL 2006	4	1	2	3++	1	2	2-	2+
Sr31/6*LMPG	*31*	CDL 2006	1	;	2	1	;1	1	1	1
W2691	*36*	BV 1999	0	3	;	0	0	0	0	0
W2691 *SrTt-1*	*36*	CDL 2006	0	3	0	0	0	0	0	0

Tester line	***Sr* gene**	**Source**	**Pathotype[a]**							
			2SA4 PSKS	**2SA36 MJQS MJRS**	**2SA55 BNGQ**	**2SA88 PTKS TTKS**	**2SA100 PJBS PJCS**	**2SA102 GFBS**	**2SA102K BFGS**	**2SA103 GDBS**
Trident	*38*	BV 2002	X-	;	;	4	;	0	;	;
McNair 701	*McN*	BV 1998	3++	3+	4	4	3	4	4	4
Kiewiet		GH 2004	;1	;1	1	;	;1	1	3++	1
Tobie	*Satu*	*Triticale*	;	0;	;	0	;	0;	0;	0;
Cns *SrTmp*	*Tmp*	CDL 2006	2	1	1	2+	1	1	1	2
Bt/Wld	*Wld-1*	CDL 2006	1	;		3+	1	3++	3+	3++

[a] Provisional North American race codes were allocated. Acme (*Sr9g*) and Renown (*Sr17*) replaced Cn*Sr9g* and Combination VII in the USA set. Variation between current and previous data resulted in uncertainty regarding the expression of *Sr17* and *Sr21*. In those cases the alternative codes are also given.

Table 3. Comparison of *Puccinia graminis* f. sp. *tritici* pathotype classification in South Africa.

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
			2SA4					**2SA36**		
5	S	S	S	S	S	S	S	S	S	S
6		S	S	S			S	S	S	S
7b	S	S	S	S	S	S	S	S	S	S
8a	S	S	S	S	S	R	R	R	S	S
8b	R		R	R	R	R		R	R	R
9a		S	S	S	S		R	S	S	
9b	S	S	S	S	S	S	S	S	S	S
9d		S	S	S	S		S	R	S	
9e	S	S	S	S	S	R	R	R	R	R
9g	R	R	R	R	R	R	R	R	R	R

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
10		S	S	S	S		R	S	S	S
11	S	S	S	S	S	R	R	S	S	S
17	S	S	S	S	S	R	R	S	S	S
21	R	R	R	R	R	R	R	R	R	R
24	R	R	R	R	R	R	R	R	R	R
27	R		R	R	R	R		R	R	R
30	S	S	S	S	S	R	R	R	R	R
31		R	R		R		R	R		R
36	R	R	R	R	R	S	S	S	S	S
38	R		R	R		R		R		
Tmp		R	R				R	R		
Wld-1		R	S				R	S		

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
McN	S		S			S		S		
Kiewiet	R		R			R		R		
Satu	R		R			R		R		
			2SA55					**2SA88**		
5	R	R	R	R		S	S	S	S	
6		R	R	R			S	S	S	
7b	R	R	R	R		S	S	S	S	
8a	S	S		S		S	S	S	S	
8b	R		R	R		S		S	S	
9a		S		S			S	S	S	
9b	R	S	R	R		S	S	S	S	
9d		S		S			S	S	S	

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
9e	R	R	R	R		S	S	S	S	
9g	R	R	R	R		S	S	S	S	
10		S		S			S	S	S	
11	S	S	S	S		S	S	S	S	
17	R	R	R	R		S	S	S		
21	R	R	R	R		R	R	R	R	
24	R	R	R	R		R	R	R	R	
27	R		R	R		R		R	R	
30	R	R	R	R		S	S	S	S	
31		R	R	R			R	R	R	
36	R	R	R	R		R	R	R		
38	R		R	R		S		S	S	

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
Tmp		R					R	R		
Wld-1		R					S	S		
McN	S					S		S		
Kiewiet	R					R				
Satu	R					R		R		
			2SA100					**2SA103**		
5	S	S	S	S	S	R	R	R	R	
6		S	S	S			R	R	R	
7b		R	S	S	S	S	S	S	S	
8a	S	S	S	S	S	S	S	S	S	
8b	R		R	R	R	R		R	R	
9a		S	S	S	S		S	S	S	

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
9b	R		S	S	S	R		R	R	
9d		S	S	S	S		S	R	S	
9e	R	R	R	R	R	R	R	R	R	
9g	R	R	R	R	R	R	R	R	R	
10		S	S	S	S		S	S	S	
11	S	R	S	S	S	R	R	R	R	
17	R	R	S	S	S	R	R	R	R	
21	R	R	R	R	R	R	R	R	R	
24	S	S	S	S	S	R	R	R	R	
27	R		R	R	R	S		S	S	
30	R	R	R	R	S	R	S	S	S	
31		R	R	R	R		R	R	R	

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
36	R	R	R	R	R	R	R	R	R	
38	R		R	R		R		R		
Tmp		R	R				R	R		
Wld-1		R	S				S	S		
McN	S		S			S		S		
Kiewiet	R					R				
Satu	R		R			R				
			2SA102					**2SA102K**		
5	R	R	R	R		R	R	R		
6		R	R	R			R	R		
7b	R	R	S	S		R	R	S		
8a	S	S	S	S		S	S	S		

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
8b	R		R	R		R		R		
9a		S	S	S			S	S		
9b	R	R	R	R		R	S	R		
9d		S	R	S			S	R		
9e	R	R	R	R		R	R	R		
9g	S	S	S	S		S	S	S		
10		S	S	S			S	S		
11	R	R	R	R		R	R	R		
17	R	R	R	R		R	R	R		
21	R	S	R			R	R	R		
24	R	R	R	R		R	R	R		
27	S	S	S	S		S		S		

***Sr* Gene**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**	**UFS[a]**	**CDL[b]**	**ARC[c]**	**Boshoff[d]**	**Le Roux[e]**
30	R	R	S	S		R	R	R		
31		R	R	R			R	R		
36	R	R	R	R		R	R	R		
38	R		R			R		R		
Tmp		R	R				R	R		
Wld-1		S	S				S	S		
McN	S		S			S		S		
Kiewiet	R					S		S		
Satu	R					R		R		

[a] University of the Free State tester lines used.

[b] Cereal Disease Laboratory tester lines used.

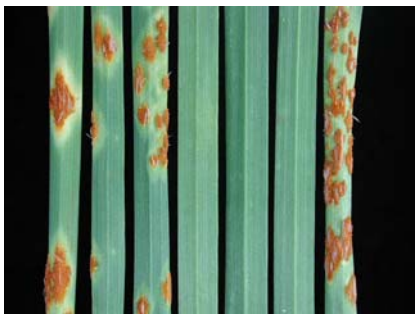
[c] Data collected from the ARC Small Grain Institute, Bethlehem.

[d] Le Roux & Rijkenberg (1987a,b) and Le Roux (1989).

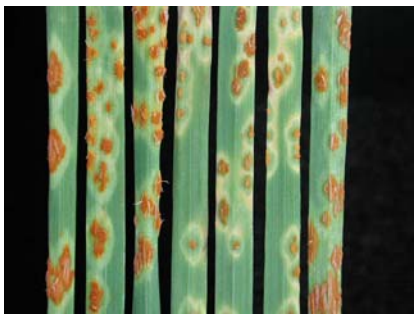
[e] Boshoff, van Niekerk & Pretorius (2000) and Boshoff *et al.* (2002).

Figure 1. Primary leaf infection types displayed by selected stem rust differential lines to pathotypes from left to right, 2SA88, 2SA100, 2SA36, 2SA102, 2SA102K, 2SA55 and 2SA4 of *Puccinia graminis* f. sp. *tritici.* The following differential lines were photographed: Reliance (*Sr5*), Marquis (*Sr7b*), Vernal (*Sr9e*), Acme (*Sr9g*), Renown (*Sr17*), Coorong (*Sr27*), Festiguay (*Sr30*), Barletta Benvenuto (*Sr8b*), Trident (*Sr30*) and Yalta (*Sr11*).

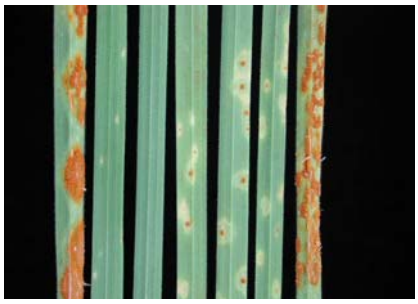
Sr5



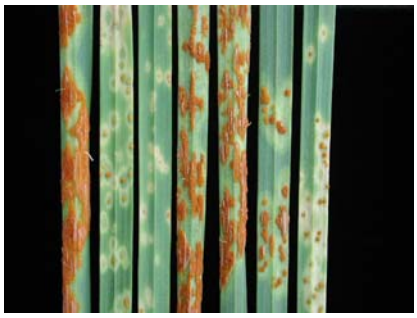
Sr7b



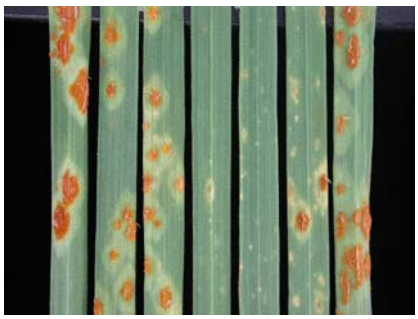
Sr9e



Sr9g

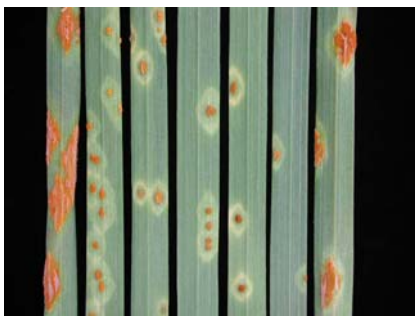


Sr17

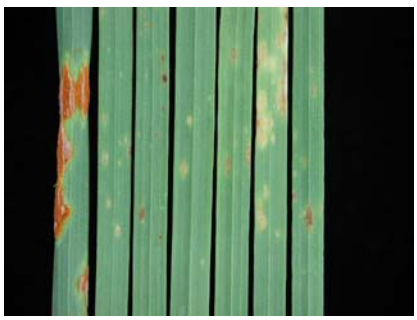


Sr27

Sr30

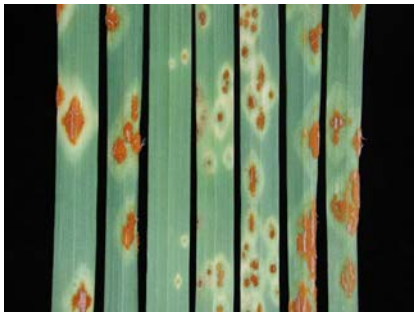


Sr8b



Sr38

Sr11



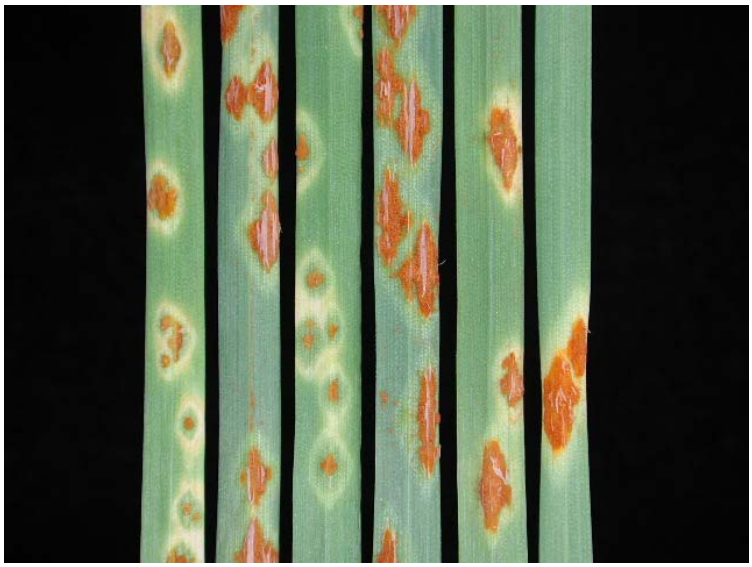


Figure 2. Variation in infection types described for *Sr9b* on stem rust differentials obtained from the University of the Free State and Cereal Disease Laboratory. From left to right: W2402 (*Sr9b*) and W2691 (*Sr9b*) infected with pathotypes 2SA102K, 2SA55 and 2SA88, respectively.

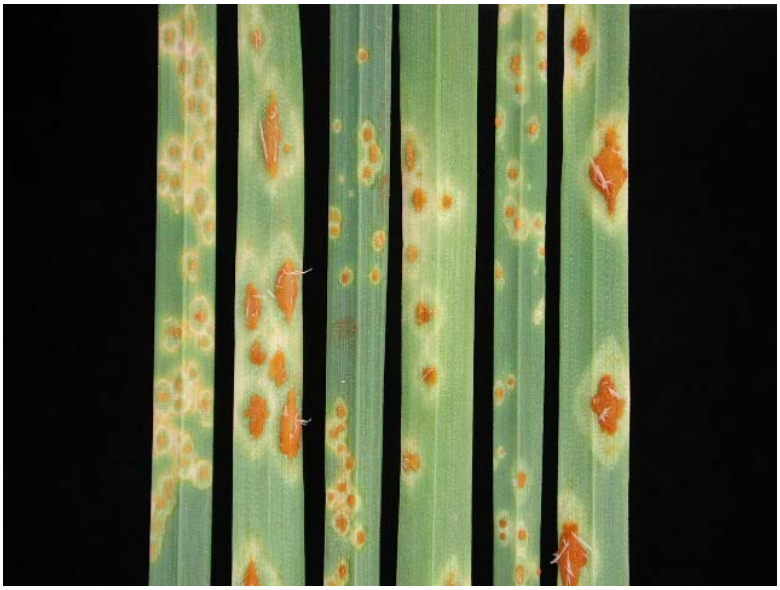


Figure 3. Variation in infection types described for *Sr21* on stem rust differentials obtained from the University of the Free State and Cereal Disease Laboratory. From left to right: Einkorn (*Sr21*) and Cns *T. mono deriv*. (*Sr21*) infected with pathotypes 2SA88, 2SA102K and 2SA102, respectively.

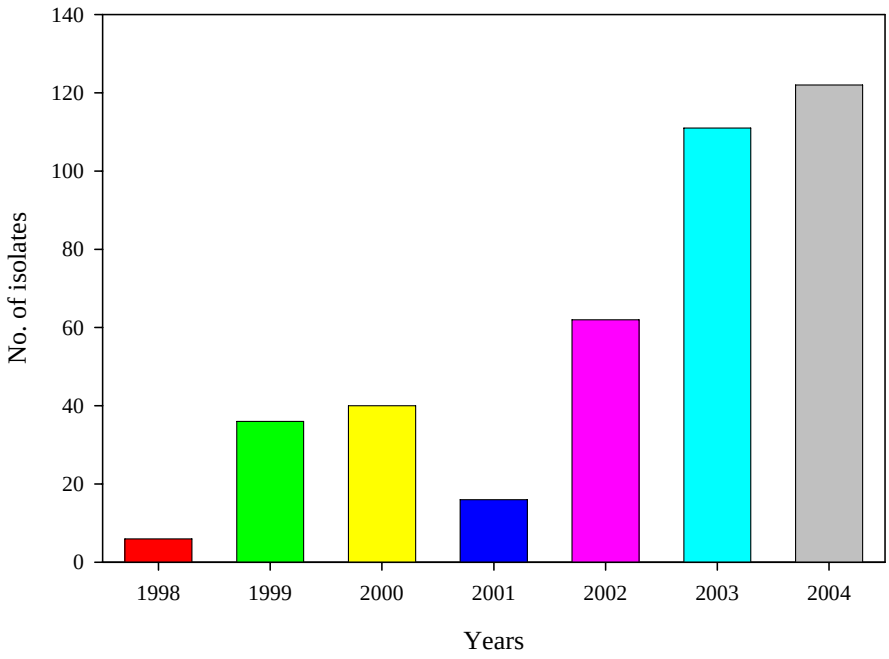


Figure 4. The gradual increase in number of stem rust isolates obtained and tested during the survey period 1998-2004.

CHAPTER 3

IN VITRO TOXICITY AND RESIDUAL EFFECTS OF SELECTED FUNGICIDES TO *PUCCINIA GRAMINIS* F. SP. *TRITICI*

3.1 ABSTRACT

The recent increase of stem rust in the Western Cape production area of South Africa has led to a renewed interest in the use of fungicides. At present only tebuconazole is registered to control stem rust in South Africa. To optimize *in vitro* testing conditions the effect of temperature on germination and germ tube growth of *Puccinia graminis* f. sp. *tritici* as well as incubation periods were evaluated. Seventy eight percent of urediniospores had germinated on water agar in petri dishes after 6 h of incubation at 20 and 25°C. Low germination rates were observed at 30°C. Twenty-nine fungicides in nine chemical classes were evaluated *in vitro* for toxicity to stem rust urediniospores. Water agar petri dish plates were amended with fungicides at concentrations ranging from 10 to 0.0001 µl active ingredient / ml medium. After incubation the percentage germination and average germ tube length were determined. Low EC_{50} (effective concentration that results in 50% inhibition) values were obtained with azoxystrobin, trifloxystrobin, kresoxim-methyl, mancozeb, azoxystrobin/difenoconazole, iprodione, chlorothalonil and hexoconazole. Fungicides such as quinoxyfen did not exhibit any toxic effect to urediniospores. When testing the effect of exposure time of stem rust urediniospores to selected fungicides, germination decreased when spores were exposed to trifloxystrobin and azoxystrobin/difenoconazole for 1 min and was completely inhibited after 30 min. Tebuconazole and flusilazole/carbendazim inhibited germination after 90 min. A shorter residual effect of trifloxystrobin occurred in wheat infected with stem rust in a glasshouse assay whereas longer efficacy periods were observed for azoxystrobin/difenoconazole and tebuconazole.

3.2 INTRODUCTION

The occurrence of stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., has increased on commercial wheat in the Western Cape over the past seasons. This increased incidence can be attributed to the widespread cultivation of susceptible cultivars, and is of concern to the South African small grain industry. Fungicide sprays have become part of the production practice of wheat producers in the Western Cape. Fungicides used for the control of wheat leaf diseases can account for more than 20% of the variable cost of wheat production in the Western Cape and are an integral part of cereal crop management in that region. According to Nel *et al.* (1999) only tebuconazole is registered for control of wheat stem rust in South Africa. However, ACDASA and CropLife South Africa list tebuconazole, triadimefon, propiconazole, and cyproconazole plus propiconazole, as registered wheat stem rust fungicides (ProCrop™ Professional software version 1.30). Limited data are available on other fungicides which, in some instances, are used extensively by producers.

Many fungicides are applied at specific growth stages and until recently, surveys have indicated that up to one-third of applications may be too late for optimal effectiveness. The severity of any disease is related to inoculum pressure, meteorological conditions and cultivar susceptibility (Cook, Hims & Vaughan, 1999). According to Milne *et al.* (2007) product choice and the timing of applications are often poor in commercial crops. Crops receiving up to three badly-timed sprays often suffer as much disease as untreated crops, suggesting that certain fungicides are applied too late or do not control the disease effectively. Research has often shown that fungicides applied during the period from flag leaf emergence to ear emergence (GS 37-59) offer the best prospects for cost-effective disease control on wheat (Bradley, 2004).

The management of stem rust through resistant cultivars is undoubtedly the most desirable means of control. However, fungicides have been used against various diseases of cereals for over 100 years. With the introduction of systemic fungicides in the 1970's, fungicides became widely used on a routine basis in cereal production. They have become established as an essential input in the growing of cereals and without them yields would be severely reduced (Dunne, 2002). Despite a progressive tightening of restrictions on chemical development and usage, such limitations can

also serve as a driving force in the discovery of new compounds (Sbragia, 1975). This is supported by the development of new fungicide classes with novel modes of action in the 1990's. These include the strobilurins, phenylpyrroles, anilinopyrimides, phenoxyquinolines and compounds that trigger defence mechanisms in the plant (Knight *et al.,* 1997).

The East African epidemics of wheat stem rust due to race Ug99 of *P. graminis* f. sp. *tritici* (Pretorius *et al.*, 2000) have stimulated a renewed interest in the search for effective and economically feasible chemicals to control this disease. Research on chemical control of stem rust has been documented, amongst others, by Mayfield (1985), Rowell (1985) and Loughman, Jayasena & Majewski (2005). Little research has recently been done to determine the direct toxic effect of fungicides to stem rust urediniospores in active lesions. The application of fungicides eliminating spores on plant surfaces, as well as through systemic activity, is beneficial from a chemical control perspective. The objective of this study was to optimize procedures for *in vitro* testing, to determine the toxic effect of fungicides labelled for use on rust diseases and to determine the efficacy of four foliar fungicides over time on wheat stem rust under controlled conditions.

3.3 MATERIALS AND METHODS

3.3.1. Rust pathogen. Inoculum of the stem rust pathotype UVPgt55 (virulent to *Sr5, 6, 7b, 8b, 9b, 9e, 11, 17, 30, 38*) was increased in a glasshouse on the susceptible cultivar Trident. Freshly collected urediniospores were used in all experiments.

3.3.2. Effects of temperature and incubation period on germination and germ tube growth of urediniospores. The effect of four temperatures, viz. 15, 20, 25 and 30°C on germination and germ tube growth of urediniospores of pathotype PgtUV55 incubated in the dark for 3, 6, 9, 12 and 15 h was examined. Water agar plates (1.5% and 9-cm diameter) and four replicates of each treatment were used. The plates were uniformly inoculated with 8 mg dry, freshly harvested urediniospores by placing them in a previously calibrated spore-settling tower. The spores were evenly dispersed by an air gun and allowed to settle for 3 min.

The inoculated plates were placed in four different growth chambers, each set at the desired temperature. The first group of 20 plates (four plates per temperature) was removed from the growth chambers after 3 h and continued until the final group was removed after 15 h. Upon removal the urediniospores were killed with lactophenol (Ellison *et al*., 1990). The percentage of germinated urediniospores was determined by microscopically counting 100 randomly selected spores on each plate. The spores were considered germinated if the tube was at least twice as long as the diameter of the spore. The length of germ tubes was measured at 20X magnification using a calibrated micrometer in the microscope eyepiece. The length of 10 germ tubes selected on each plate was measured.

3.3.3. Fungicide experiments

Twenty nine fungicides, either registered, in experimental phase, or mentioned as having activity to rust diseases, irrespective of the host plant, were evaluated.

- **EC_{50} determination**

Water agar was autoclaved and allowed to cool to 50°C before a fungicide was incorporated into the medium. For each fungicide concentrations of 0, 0.01, 0.1, 1 and 10 µg active ingredient per ml of water agar were tested in 9-cm diameter petri dishes. All treatments were replicated four times. The pH of each fungicide-amended medium was recorded.

Using a settling tower, the fungicide-amended petri dishes were inoculated with spores of pathotype UVPgt55 as described earlier. The inoculated petri dishes were incubated in the dark for 6 h at 20°C in a growth chamber. After incubation the urediniospores were killed and the percentage germination and germ tube length were recorded for each fungicide treatment. The EC_{50} values for each fungicide were determined according to Bliss (1935). For some compounds, e.g. the strobilurin fungicides, a lower concentration of 0.0001 µl a.i / ml agar was included.

- **Germination response to fungicide exposure**

Five fungicides were used to determine the effect of urediniospore survival after exposure for certain time intervals. The fungicides, namely Amistar Top® (azoxystrobin/difenoconazole), Punch Xtra® (flusilazole/carbendazim), Folicur®

(tebuconazole), Twist® (trifloxystrobin) and Legend® (quinoxyfen), and a water control, were included. Treatments were replicated four times. The fungicides were sprayed at recommended rates [azoxystrobin/difenoconazole (100/62.5 g a.i. ha^{-1}), flusilazole/carbendazim (50/100 g a.i. ha^{-1}), tebuconazole (187.5 g a.i. ha^{-1}), trifloxystrobin (50 g a.i. ha^{-1}) and quinoxyfen (125 g a.i. ha^{-1})] onto sporulating wheat seedlings. After 1, 5, 10, 30, 60, 90 and 120 min intervals the spores were collected with a cyclone collector. The collected spores were placed in 2 ml eppendorf tubes containing distilled, sterilized water. The tubes were centrifuged for 10 sec before collection of the supernatant. The process was repeated twice before the spores were pipetted onto water agar petri dishes. The inoculated petri dishes were placed in the dark in a growth chamber for 6 h at 20°C. The spores were killed by lactophenol after incubation and percentage germination and germ tube length were determined. The experiment was conducted twice.

- **Residual effects**

The wheat cultivar Trident, which is susceptible to pathotype UVPgt55, was grown in a sterilized soil-peat moss mixture in 9-cm diameter pots in a disease-free glasshouse cubicle. After emergence seedlings were thinned to ten plants per pot. Fourteen days after planting seedlings were sprayed with tebuconazole, flusilazole/carbendazim, trifloxystrobin and azoxystrobin/difenoconazole and a water control. Trade names, formulations and recommended rates are listed in Table 1. Applications were done with a pressurized knapsack sprayer at 200 kPa using hollow cone nozzles, recommended rates and water volumes simulating 300 ℓ ha^{-1}. The fungicide-treated plants were kept in a glasshouse cubicle at 20± 4°C and fertilized weekly by a water-soluble solution of 2 g/ℓ Multifeed®.

The first sets of plants were inoculated 3 h after fungicide applications. This was followed by consecutive inoculations 7, 14, 21 and 28 days after fungicide treatment. The plants (four pots per treatment) were inoculated with pathotype UVPgt55 using 8 mg of freshly collected urediniospores, suspended in light mineral oil. Inoculations were done in an enclosed inoculation booth. After drying for 2 h in an air-conditioned room, inoculated plants were placed in the dark in a dew chamber at 20±1°C and >96% relative humidity for 16 h. Upon removal from the chamber plants were dried down in a growth chamber at 20°C for 3 h before placement in a glasshouse cubicle at 20±4°C. Daylight was supplemented with cool-white

fluorescent tubes emitting photosynthetic active radiation of 120 μE/m^2/s for 14 h each day.

Fourteen days after inoculation plants were evaluated for stem rust incidence (percentage of plants showing symptoms), total number of pustules observed on the second leaf of each plant, infection type (0 to 4 scale, Stakman, Steward & Loegering, 1962) and pustule size. The length (mm) of 10 randomly selected pustules per leaf was measured using a digital caliper. The experiment was arranged as a randomized complete block with four replications. The trial was conducted twice.

3.3.4. Statistical analysis

Data were analyzed for variance using the statistical software NCSS 2000 for Windows. Means were compared using Tukey's least significant difference test (Steel & Torrie, 1980).

3.4 RESULTS AND DISCUSSION

3.4.1. Effects of temperature and incubation period on germination and germ tube growth of urediniospores

The main objective for the first experiment was to determine the optimum temperature and dew period for *in vitro* germination of stem rust urediniospores. Significant ($P \leq 0.05$) effects due to temperature, duration of incubation period and temperature x duration for germination were detected. Germination varied from 78.7% (20°C, 6 h incubation period) to 9.7% (30°C, 12 h incubation period). As temperature increased from 15 to 30°C, spore germination increased to a maximum of 78.3% at 20°C and then declined (Figure 1). Spore germination was not significantly affected by the duration of incubation. Germination was initiated at 3 h and reached a maximum after 6 h.

Results demonstrated that urediniospores of *P. graminis* f. sp. *tritici* germinated over all temperatures tested but that the process was sub-optimal at 15 and 30°C. According to McIntosh, Wellings & Park, (1995) stem rust develops successfully within the range 18-30°C. Wiese (1987) stated that stem rust develops optimally near 20°C and is seriously hampered below 15°C and above 40°C. Spore

germination *in vitro* tests done by Rowell (1985) showed that the optimum temperature for stem rust incubation was at 18°C for 24 h. In general similar results were obtained for germ tube growth in response to temperature and incubation period (data not shown). According to the present data, the optimum incubation period for stem rust urediniospores to germinate was 6 h and this time was employed in further experimentation.

3.4.2. EC_{50} determination

Germination of stem rust urediniospores was significantly affected by the fungicides tested and by the concentration of active ingredient used (Table 2). The EC_{50} values for germination and germ tube length differed significantly between fungicides ($P \leq 0.05$). There was also a significant difference between concentrations for each fungicide tested.

Of the 29 fungicides tested, azoxystrobin, trifloxystrobin, kresoxim-methyl, mancozeb, azoxystrobin/difenoconazole, iprodione, chlorothalonil and hexoconazole inhibited or almost restricted germination. EC_{50} values ranged from 0.001 to 0.004 µg a.i./ mℓ for the strobilurins, 0.118 to 20.944 for the triazoles, 0.002 to 199.1 for seed treatment fungicides, and 0.053 to 1060.679 µg a.i./ mℓ for other compounds tested. Some fungicides, e.g. cyproconazole, triticonazole, quinoxyfen and the experimental seed treatment Jockey Flex, gave high EC_{50} values for spore germination, while only quinoxyfen and Jockey Flex responded similarly with high EC_{50} values for germ tube length. The coefficient of variability for the latter two fungicides was exceptionally high, indicating a lack of accuracy in this method when using ineffective fungicides.

The five strobilurin fungicides and the mancozeb seed treatments were fungicidal and killed the urediniospores at low concentrations. Eight fungicides from the triazole group, four fungicides from other groups and two experimental fungicides displayed low EC_{50} values. With the exception of triticonazole and Jockey Flex, the seed treatment chemicals were effective. Despite their efficacy, it is unlikely that seed treatments will be effective in controlling stem rust infections late in the season. Due to their toxicity the strobilurin fungicides had to be tested at a concentration lower than the originally planned 0.01 µg active ingredient per ml agar to determine their EC_{50} values. According to Dunne (2002) the strobilurin group is excellent inhibitors of spore germination and the present investigation supported this statement.

3.4.3. Germination response to fungicide exposure

The experiments did not differ significantly and data were pooled. Germination was affected differentially when urediniospores were exposed to five fungicides for different lengths of time. The interaction between fungicide and time was significant ($P \leq 0.05$) and fungicides influenced germination significantly. Over the duration of this experiment, the effects of trifloxystrobin and azoxystrobin / difenoconazole were similar, and the effects of tebuconazole and flusilazole / carbendazim were similar. Quinoxyfen did not have a toxic effect on stem rust urediniospores and gave results similar to the control (Figure 2).

Urediniospores were killed by trifloxystrobin and azoxystrobin / difenoconazole when exposed for 30 min (Figure 2). In the tebuconazole and flusilazole / carbendazim treatments spore germination decreased gradually, completely inhibiting germination after 120 min exposure. *In vitro* fungicide efficacy has been evaluated by testing the ability of a compound to inhibit spore germination. The information is valuable in identifying fungicides that kill non-germinating urediniospores on contact. Therefore, four fungicides were selected according to their EC_{50} values determined in the first toxicity experiment. These fungicides were fungicidal to urediniospores and were presumably able to penetrate the spore wall to accumulate to a lethal dose.

Trifloxystrobin and azoxystrobin/difenoconazole appeared to quickly enter the spore. Significant reductions in germination were observed after exposure for only 1 min reaching zero germination after 30 min of fungicide contact. In contrast, uptake of tebuconazole and flusilazole/carbendazim into the urediniospores took longer (Figure 2). The fungicide quinoxyfen, which was included as an ineffective control, did not have a toxic effect on the urediniospores and gave results similar to the water control. The identification of fungicides that kill quiescent urediniospores on contact can serve several purposes. These compounds will be useful in reducing spore viability in sporulating uredinia, thereby slowing disease progress within the crop as well as the potential development of fungicide resistant isolates (Mueller, Jeffers & Buck, 2005). The efficacy of fungicides in the triazole group against stem rust was expected.

Previously Rowell (1981) had indicated that triadimefon was effective for control of wheat stem rust epidemics on spring wheat in the North-central United States. Likewise, due to their fungitoxic effect on urediniospores, the strobilurins appear to have potential for stem rust control. At present no strobilurin fungicide is registered on wheat in South Africa and further field experimentation may prove useful. Questions often arise if *in vitro* exposure of rust mycelia to fungicides is similar to what can be expected in the host. The external concentration required for a lethal dose is not always an accurate measure of the actual efficacy of a chemical. According to Rowell (1985) fungicidal action of a compound is a function of both its ability to be taken up in the host plant and its inherent toxicity.

3.4.4. Residual effects

In an analysis to test similarity of data in the two independent experiments, the effect of fungicides on pustule length (P=0.0005) and pustule size (P=0.0003) was significant. The data are therefore presented separately (Figures 3-6).

The incidence of stem rust ranged from no visual infection (tebuconazole and azoxystrobin/difenoconazole) to an average of 90% of plants infected (trifloxystrobin and water control). Infection type differed between treatments over time. High infection types (3 and 4) were found for the control and trifloxystrobin 7 days after treatment while the treatment with flusilazole/carbendazim gave infection types of 1C and 2+3 on individual plants, 21 days after treatment.

No significant difference was found for pustule size and number of pustules between the control and trifloxystrobin after seven days. Therefore, it can be assumed that trifloxystrobin has a low residual effect on plants infected with stem rust. After seven days, a slow increase in pustule number and size was found on plants treated with the fungicide flusilazole/carbendazim. Compared to the control, significantly lower values were found for pustule size and pustule number even after 28 days. In both experiments no infection was found on plants treated with tebuconazole. Low infection levels were found with azoxystrobin/difenoconazole after 28 days in the first experiment. Tebuconazole showed a high residual effect and provided protection throughout the duration of the experiment.

From this study, it can be assumed that fungicides belonging to the triazole group have a longer residual effect in the plant than the fungicides from the strobilurin group. When combining the data from the *in vitro* and glasshouse experiments, the

strobilurin fungicides have an excellent ability to kill stem rust urediniospores on contact but showed a shorter residual effect in plants. The triazole fungicides took longer to kill stem rust urediniospores but exhibited a longer residual effect. Triazole derivatives have exhibited both fungicidal and plant growth regulating properties and are inhibitors of gibberellin biosynthesis (Goa, Hofstra & Fletcher, 1988).

A promising formulation was observed for the triazole and strobilurin combination. The azoxystrobin/difenoconazole has also shown promising results on rust diseases on maize (F.J. Kloppers, Pannar Research, Greytown, personal communication). According to Mueller *et al.* (2005), azoxystrobin is known to be actively taken up by the plant cells and is upwardly systemic where it can provide protection from certain pathogens for up to 8 weeks. Continual pressure for improved safety with agricultural chemicals will provide a strong impetus for discovering alternative types of activity. The demand for new innovative chemicals is very strong, but chemicals alone are not the answer to reduce crop losses. An integrated programme includes sanitation, good cultural practices and resistant varieties, all linked to accurate disease forecasting and sensible applications of chemicals (Sbragia, 1975).

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Table 1. Fungicides registered or in experimental phase used that were evaluated for toxicity to stem rust urediniospores

Fungicide category	Active ingredient	Trade name	Formulation	Rec. rate	Labeled for rust
Strobilurin	Azoxystrobin	Amistar®	250 SC	300ml/ha	Yes
	Kresoxim-methyl	Stroby®	50 WG	15g/ha	No
	Trifloxystrobin	Flint®	50 WG	15g/ha	No
	Trifloxystrobin	Twist®	500 SC	100ml/ha	Yes
Strobilurin / DMI triazole	Azoxystrobin/Difenoconazole	Amistar Top®	200/125	500ml/ha	Yes
DMI triazole	Bitertanol	Baycor®	300 EC	650ml/ha	Yes
	Bromuconazole	Granit®	100 EC	700ml/ha	Yes
	Cyproconazole	Alto®	100 SL	400ml/ha	Yes
	Difenoconazole	Score®	250 EC	350ml/ha	Yes
	Epoxiconazole	Opus®	125 SC	800ml/ha	Yes
	Flusilazole	Capitan®	250 EW	400ml/ha	Yes
	Hexaconazole	Anvil®	50 SC	480ml/ha	Yes
	Propiconazole	Tilt®	250 EC	500ml/ha	Yes
	Tebuconazole	Folicur®	250 EW	750ml/ha	Yes
	Triadimefon	Bayleton®	250 EC	500ml/ha	Yes
	Triadimenol	Baytan®	150 FS	150g/100kg	No
	Triticonazole	Flite®	200 FS	120g/100kg	Yes
DMI piperazine	Triforine	Denarin®	190 EC	150ml/ha	Yes

Table 1 (cont) Fungicides registered or in experimental phase used that were evaluated for toxicity to stem rust urediniospores

Fungicide category	Active ingredient	Trade name	Formulation[a]	Rec. rate[b]	Labeled for rust
DMI triazole/ benzimidazole	Flusilazole / carbendazim	Punch Xtra®	125 / 250	400ml/ha	Yes
Dihiocarbamate	Mancozeb	Dithane®	800 WP	150g/100kg	Yes
	Mancozeb	Ifax®	800 DS	160g/100kg	Yes
Phthalimide	Chlorothalonil	Bravo®	720 SC	190ml/ha	Yes
Dinitrophenyl	Dinocap	Karathane®	350 EC	50ml/ha	No
Multi site	Quinoxyfen	Legend®	250 SC	500ml/ha	No
Anilide	Oxycarboxin	Plantvax®	200 EC	1500ml/ha	Yes
Dicarboximide	Iprodione	Rovral Flo®	255 SC	200ml/ha	No
Experimental[c]	-	Jockey Flex®	167 FS	450ml/100kg	Yes experimental
	-	JAU 6478	100 FS	100ml/100kg	Yes experimental
	-	JAU 6478 / HWG 1608	250 EC	400ml/ha	Yes experimental

[a] Percentages of active ingredients in commercial products formulated as powder of dry seed treatment (DS), emulsifiable concentrate (EC), emulsion in oil or water (EW), flowable concentrate for seed treatment, suspension concentrate (SC), soluble concentrate (SL), water dispersible granule (WG) and wettable powder (WP) (Nel, Krause, Ramautor and van Zyl, 1999).

[b] Recommended use (as per chemical company) of fungicides per hectare or as seed treatment per 100kg of seed.

[c] Fungicides still in experimental phase.

Table 2. The EC_{50} values for germination and germ tube length, with the average CV for each fungicide and rankings according to their performance.

Fungicide category	Active ingredient	Germination			Germ tube length (µm)		
		EC_{50}	**CV**	**Rank**	**EC_{50}**	**CV**	**Rank**
Strobilurin	Azoxystrobin	0.002	0.0	6	0.002	0.0	5
	Kresoxim-methyl	0.001	0.0	3	0.001	0.0	3
	Trifloxystrobin	0.001	0.0	4	0.001	0.0	4
	Trifloxystrobin	0.001	0.0	2	0.001	0.0	1
Strobilurin / DMI triazole	Azoxystrobin/Difenoconazole	0.003	24.7	7	0.003	24.7	7
DMI triazole	Bitertanol	22.972	9.5	24	2.210	4.1	23
	Bromuconazole	6.835	11.9	22	1.198	7.9	22
	Cyproconazole	1733.460	30.8	27	11.329	15.8	25
	Difenoconazole	0.497	3.3	18	0.154	11.5	16
	Epoxiconazole	0.569	7.1	20	0.225	8.9	21
	Flusilazole	0.520	7.8	19	0.177	11.6	18
	Hexaconazole	0.104	11.2	10	0.034	5.1	10
	Propiconazole	18.973	7.3	23	23.924	19.5	26
	Tebuconazole	0.312	11.6	15	0.131	5.7	14
	Triadimefon	0.164	5.8	12	0.043	10.1	11
	Triadimenol	28.619	23.4	25	25.569	36.1	27

Fungicide category	Active ingredient	Germination			Germ tube length (µm)		
		EC_{50}	CV	Rank	EC_{50}	CV	Rank
	Triticonazole	666.100	69.9	26	2.343	2.7	24
DMI piperazine	Triforine	0.415	6.0	17	0.142	3.6	15
DMI triazole/ benzimidazole	Flusilazole / carbendazim	0.310	1.4	14	0.193	2.1	19
Dihiocarbamate	Mancozeb	0.002	0.0	5	0.002	0.0	6
	Mancozeb	0.001	0.0	1	0.001	0.0	2
Phthalimide	Chlorothalonil	0.063	11.4	9	0.032	6.4	9
Dinitrophenyl	Dinocap	0.171	11.6	13	0.061	4.9	12
Multi site	Quinoxyfen	1437491.9	172.0	28	320772.95	92.7	28
Anilide	Oxycarboxin	0.325	3.5	16	0.163	2.2	17
Dicarboximide	Iprodione	0.052	4.0	8	0.026	4.4	8
Experimental***	Jockey Flex	233784655	102.3	29	30371053	129.5	29
	JAU 6478	0.116	6.0	11	0.096	6.0	13
	JAU 6478 / HWG 1608	0.629	40.7	21	0.216	9.7	20

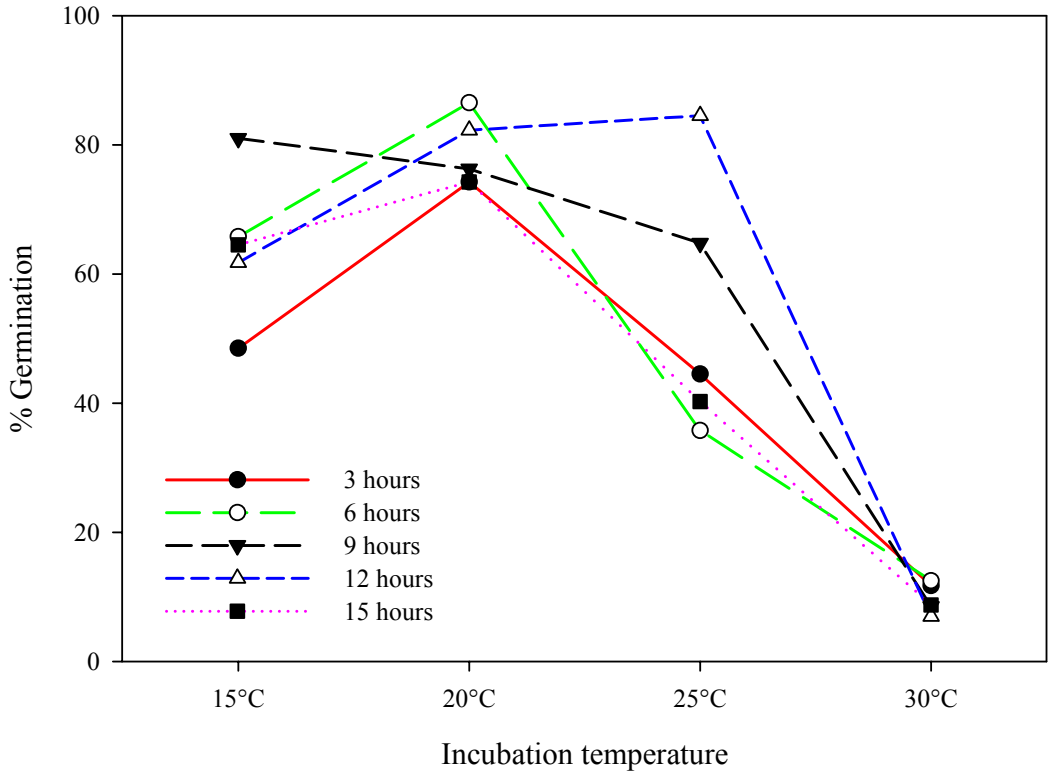

Figure 1. *In vitro* germination of urediniospores of *Puccinia graminis* f. sp. *tritici* as affected by incubation temperature and incubation time. The Tukey (P=0.05) value for comparison of means is 6.43.

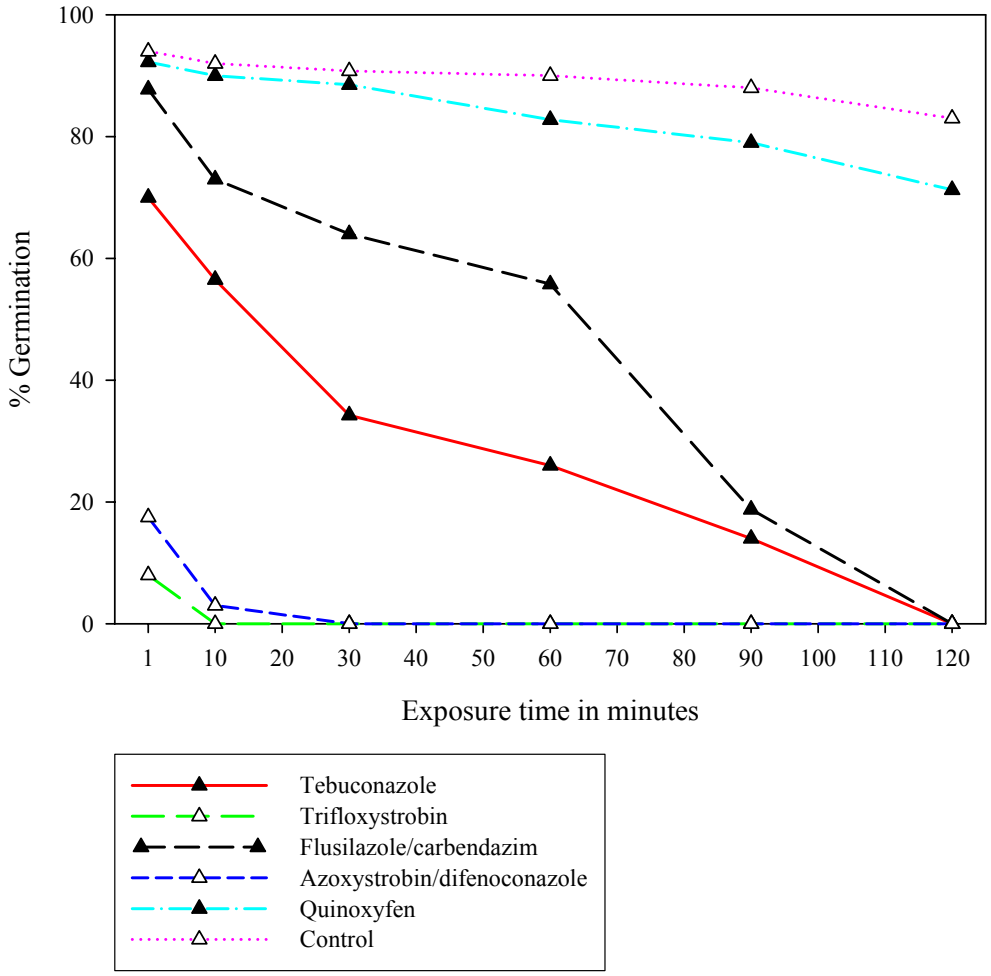

Figure 2. The percentage germination of freshly collected urediniospores of *Puccinia graminis* f. sp. *tritici* following exposure to fungicides for different time periods. The Tukey (P=0.05) value for comparison of means is 6.14.

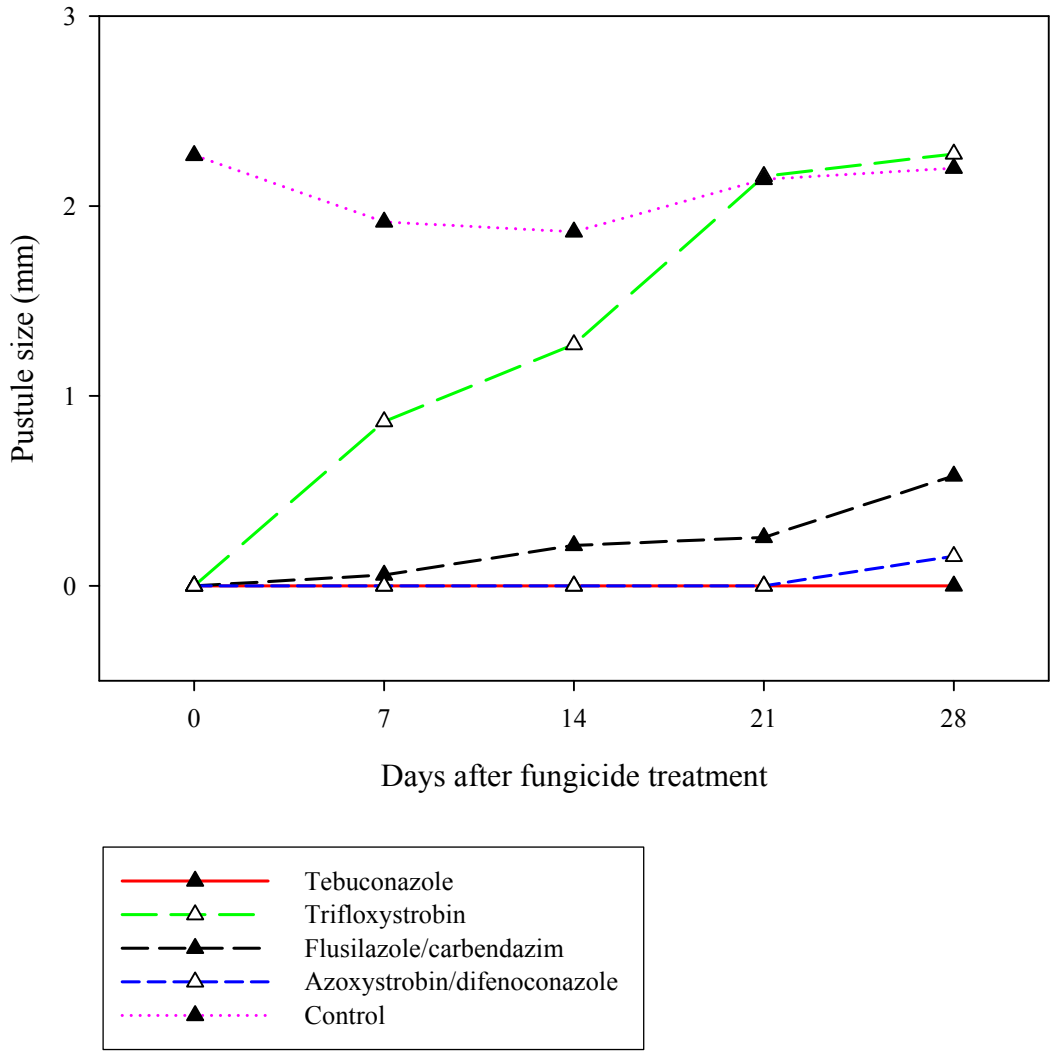

Figure 3. The mean pustule size of stem rust on wheat plants sprayed with fungicides and inoculated at different time periods after fungicide application in experiment 1. The Tukey (P=0.05) value for comparison of means is 0.42.

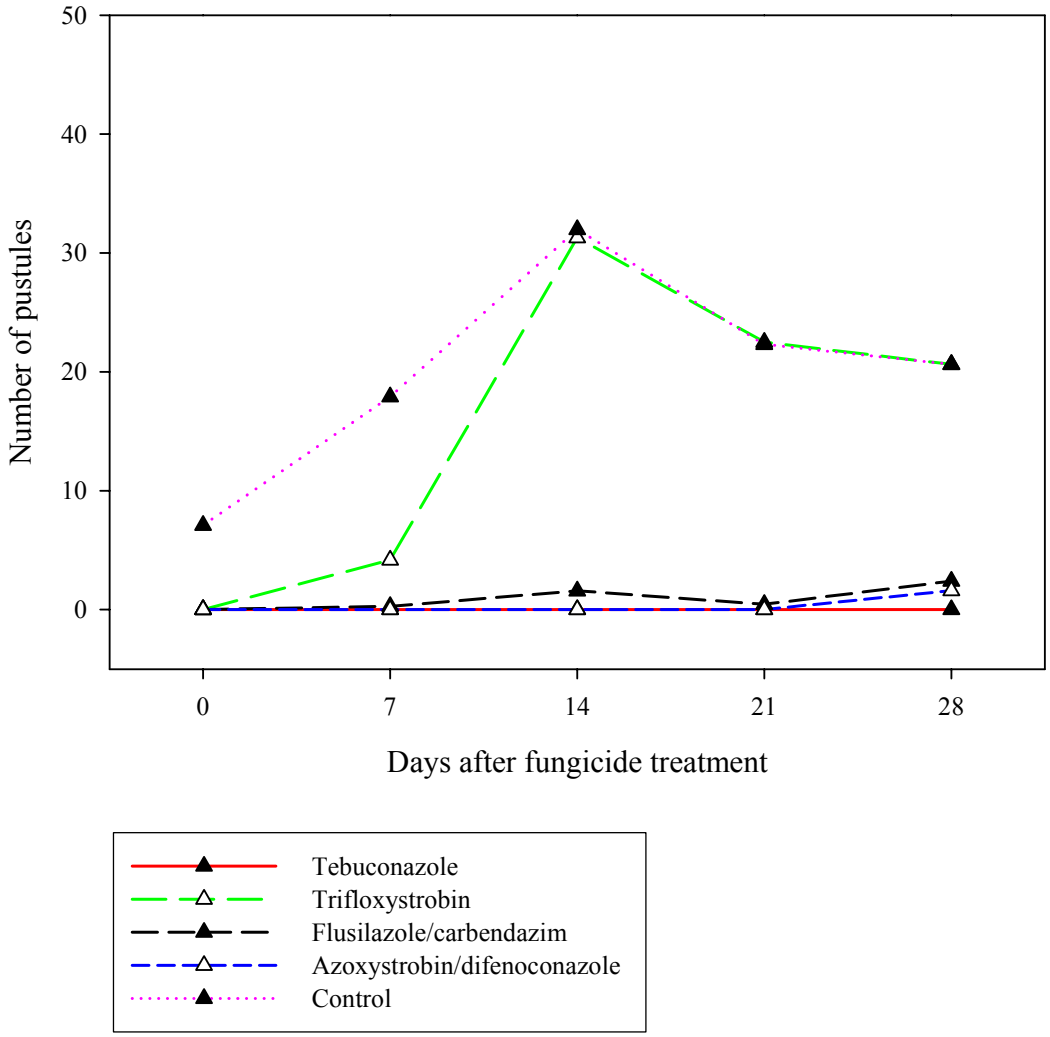

Figure 4. The mean number of stem rust pustules on the 2nd leaf of wheat plants sprayed with fungicides and inoculated at different time periods after fungicide application in experiment 1. The Tukey (P=0.05) value for comparison of means is 7.86.

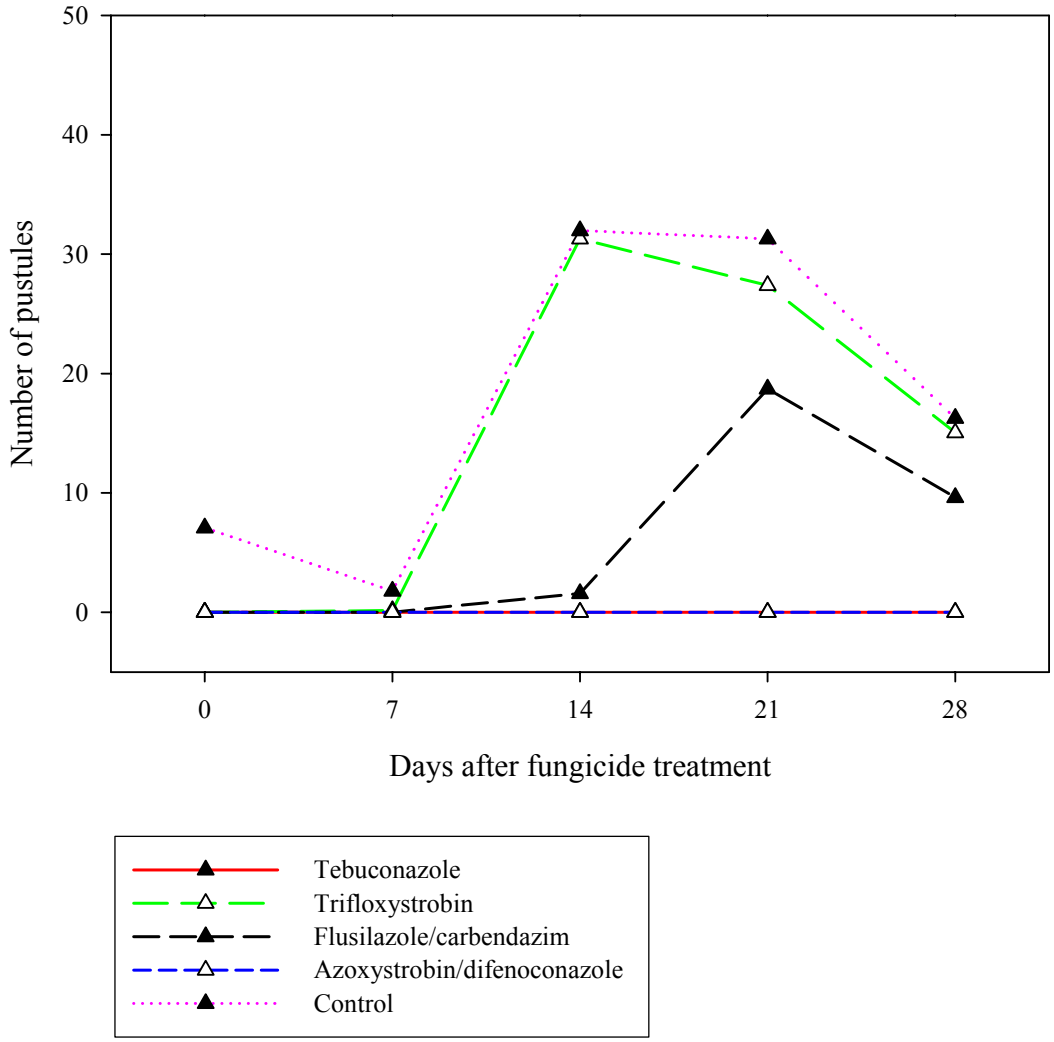

Figure 5. The mean number of stem rust pustules on the 2nd leaf of wheat plants sprayed with fungicides and inoculated at different time periods after fungicide application in experiment 2. The Tukey (P=0.05) value for comparison of means is 5.93.

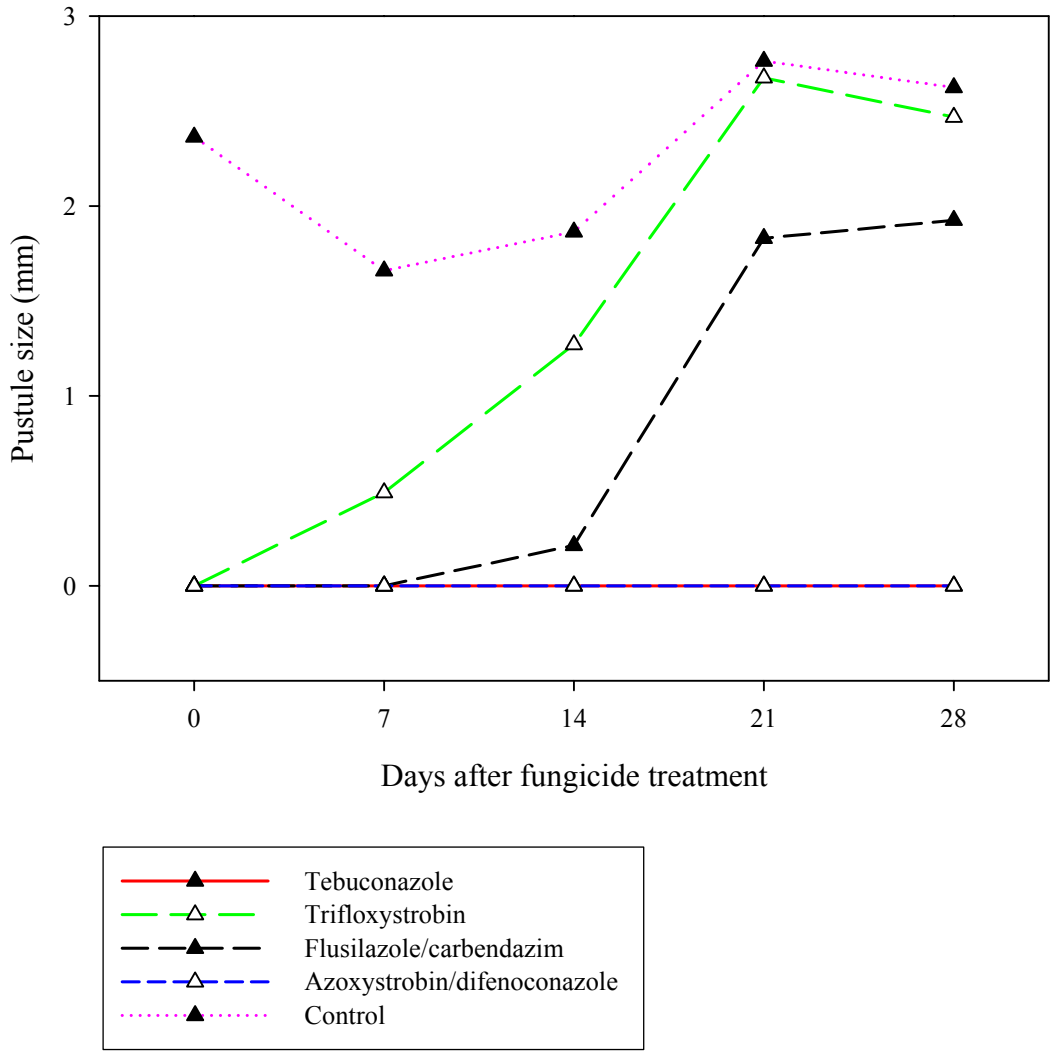

Figure 6. The mean pustule size of stem rust on wheat plants sprayed with fungicides and inoculated at different time periods after fungicide application in experiment 2. The Tukey (P=0.05) value for comparison of means is 0.33.

CHAPTER 4

THE EFFECT OF STEM AND LEAF RUST ON YIELD OF BREAD WHEAT

4.1 ABSTRACT

Rusts remain the most important diseases of wheat worldwide and localized epidemics have become more frequent in many regions. The release of rust-susceptible cultivars in South Africa is of great concern and therefore research was conducted to determine if chemical control is an economically viable method to control rust diseases. Fungicide field trials were conducted over two years at Greytown, South Africa to determine the effect of rust diseases on yield and test weight. Information on the efficacy of different foliar treatments in controlling these diseases was obtained. The wheat cultivar SST 88 was used in both experiments and four fungicides and three application methods were tested. Although the emphasis was on stem rust, it was not possible to eliminate leaf rust from the field trials. In 2005, 92% of the yield reduction experienced was contributed by leaf rust infection whereas in 2006, 32% of losses could be attributed to this disease. Stem rust on the other hand contributed 8% to losses in 2005 and 68% in 2006. Mean yield increase resulting from fungicide applications ranged from 36% to 45% over the two years. With a flag leaf treatment the mean yield increase per plant was 21% compared to 43% with a multiple-spray treatment in 2005. The highest yield increase was obtained with tebuconazole resulting in a 58% gain. Fungicide application increased test weight by 29% over the control treatment. No significant differences were found between application techniques.

4.2 INTRODUCTION

Rusts remain important diseases of wheat (*Triticum aestivum* L.) worldwide because of their wide distribution, capacity to form new pathotypes that can attack previously resistant cultivars, ability to move long distances, potential to develop rapidly under optimal environmental conditions, and economic impact. Stem rust (*Puccinia*

graminis Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn.) can cause total losses in grain yield of wheat (Mayfield, 1985).

South African regions historically prone to wheat stem rust, and where localised epidemics have been more frequent, are the Western Cape production areas. Stem rust epidemics in South Africa were last documented in 1985 when *Sr24* virulence was found (Le Roux, 1985; Le Roux & Rijkenberg, 1987a,b). The increased frequency of the disease since 2000 has been associated with the cultivation of stem rust susceptible cultivars and the occurrence of pathotype 2SA88 (Boshoff, Pretorius, Van Niekerk & Komen, 2002).

Due to the comparative rarity of stem rust, effects of fungicide control on yield reduction are poorly understood. Treatment decisions should ideally be supported by disease thresholds, either formalised or developed by crop managers through practical experience. In farm practice, the proportion of yield that is due to fungicide treatment is not usually known, and the success of spray decisions is often judged by the level of disease later in the season (Paveley *et al.*, 1997). Paveley *et al.* (2001) quantified the reduction in foliar disease severity in wheat by fungicides. They showed that fungicides delayed and reduced epidemic onset and rate, and that the magnitude of the effect was dependent on both spray timing and dosage.

Reliable methodology to estimate crop losses could enable the producer to weigh the cost of a control programme against the benefits of yield protection (Calpouzos *et al.,* 1976). The potential threat of pathotype Ug99 (Pretorius *et al.*, 2000) has renewed interest in stem rust impact and control. The objective of this chapter was to assess the damage potential of stem rust on a current spring wheat cultivar.

4.3 MATERIALS AND METHODS

In 2005 and 2006 field trials were established at Redgates, Pannar Experimental Farm, Greytown, South Africa. The test cultivar was SST 88, a popular spring wheat cultivar grown in the Western Cape. Stem rust epidemics were created by injecting fresh urediniospores of pathotype UVPgt55 into the whorls of susceptible wheat plants before the booting stage and by applying spores in a water suspension onto rust spreader rows. These spreader rows were grown between experimental plots and in

pathways perpendicular to plots. Supplementary overhead sprinkler irrigation was supplied as needed.

4.3.1. 2005 Experiment

The cultivar SST 88 was planted in 1-m rows during June. The spacing between rows was 90 cm. The experiment was designed to determine yield on a single plant basis as described by Campbell & Madden (1990). Treatments included a water control, one fungicide spray with tebuconazole at the flag leaf stage and multiple sprays with tebuconazole to serve as an uninfected control. The fungicide was applied with a knapsack sprayer fitted with a cone nozzle and water volumes of approximately 300 ℓ ha^{-1}.

Disease assessments for leaf and stem rust were carried out on tagged plants on three occasions, namely at decimal growth stages 45, 53 and 71. The percentage disease severity on flag leaves and stems of 140 plants per treatment, using a modified Cobb scale (Peterson, Campbell & Hannah, 1948), was determined. Area under the disease progress curve (AUDPC) was calculated.

At maturity, each tagged plant was harvested individually and yield and thousand kernel mass were determined. Thousand kernel mass was extrapolated from the mass of 100 kernels per plant.

4.3.2. 2006 Experiment

The cultivar SST 88 was space-planted in a randomized, split-plot design with five treatments (four fungicides and one water control), three application techniques and four replications. Each plot consisted of two 2-m rows. Fungicides served as main plots and application technique as sub plots. The fungicides tebuconazole (187.5 g a.i. ha^{-1}), trifloxystrobin (50 g a.i. ha^{-1}), flusilazole/carbendazim (50/100 g a.i. ha^{-1}) and azoxystrobin/difenoconazole (100/62.5 g a.i. ha^{-1}) were evaluated. For unregistered fungicides, the rates applied were recommended by the respective chemical company. The fungicides were applied in single applications at growth stage 61, using a knapsack sprayer and water volumes of approximately 300 ℓ ha^{-1}. Fungicides were applied either on the base of the stems, the flag leaf canopy only, and on both the stems and flag leaves.

Disease assessments were carried out on whole plots at the time of application followed by two assessments at 14-day intervals. The data were used to calculate

AUDPC for each treatment. At maturity the number of plants per row was determined and two rows per treatment were harvested. Seed were air-dried, cleaned and weighed to determine grain yield. Yield obtained from each plot was used to determine the thousand kernel mass.

4.3.3. Statistical analysis

AUDPC values calculated for rust development over time, grain yield and thousand kernel mass data obtained in the different experiments in different years, were analyzed for variance using the statistical software NCSS 2000 for Windows. Means were compared using Tukey's test (P=0.05) (Steel & Torrie, 1980).

4.4 RESULTS

The occurrence of stem and leaf rust infections and the onset of epidemics differed from year to year.

4.4.1. 2005 Experiment

Natural leaf rust infection reached epidemic proportions on SST 88 early in the season. The first leaf rust symptoms were observed in mid-August at the time of the first application of tebuconazole. No stem rust symptoms were observed at that stage. When the flag leaf treatments were applied, two weeks after the first spray, no leaf rust was observed on the multiple spray treatment plots whereas severity varied between 40 to 80% in the other treatments. Only traces of stem rust were observed at that time. After six weeks premature leaf necrosis was noted on the control treatment and 50% of the stems were rusted. The mean stem rust infection on the single flag leaf treatment was 20%, whereas no rust symptoms were detected in the multiple spray treatment.

Leaf rust infection resulted in high AUDPC values as opposed to stem rust for which a lower AUDPC was calculated (Figure 1.) The single flag leaf application reduced the AUDPC for leaf and stem rust by 32% and 39%, respectively. In a stepwise regression analysis it was shown that leaf rust contributed 92% of the reduction in yield.

Yield per plant was significantly higher following fungicide treatments which also differed statistically ($P \leq 0.05$). Compared with the control, the mean yield increase per plant was 21% following the flag leaf treatment, and 43% with the multiple spray treatment (Figure 2).

Test weight differed statistically ($P \leq 0.05$) between treatments. The test weight obtained with the multiple spray treatment was significantly higher ($P \leq 0.05$) than those obtained for the single flag leaf treatment and the control. Compared to the control, test weight for the flag leaf and multiple spray treatments increased by 18% and 33%, respectively (Figure 3).

4.4.2. 2006 Experiment

Only trace stem rust symptoms were observed during the application of the fungicides at growth stage 59. Leaf rust symptoms appeared about two weeks later on the control plots. Four weeks after fungicide application, stem rust severity varied between 5 and 20% for sprayed plots, and between 40 and 60% for the unsprayed plots. The mean leaf rust severity was 10% for the treated plots and 70% for the untreated plots.

The mean stem rust AUDPC for the fungicide treatments was 53% lower than the control whereas the mean leaf rust AUDPC was reduced by 68% (Figure 4). Tebuconazole reduced the AUDPC for stem rust and leaf rust by 83% and 89% respectively, whereas flusilazole/carbendazim reduced the incidence of the rusts by 37% and 61%. Differences occurred between the application treatments. Compared to the untreated control, fungicides applied to the stems reduced the total AUDPC by 42%. The flag leaf, and combined flag leaf and stem application, reduced AUDPC by 41% and 48% respectively (Figure 5). In contrast to 2005, the R^2 values showed that stem rust caused 68% yield reduction.

Over application positions, yield increased by 58% for tebuconazole followed by trifloxystrobin (40%), flusilazole/carbendazim (27%), and azoxystrobin/ difenoconazole (20%) (Figure 6). The yield did not differ statistically ($P \leq 0.05$) between application positions.

The test weight was affected by stem and leaf rust infection and increased by 29% in fungicide applications. Over application techniques, the test weight increased by 42% for tebuconazole followed by trifloxystrobin (25%), azoxystrobin/

difenoconazole (25%) and flusilazole/carbendazim (24%) (Figure 7). The arrangement of plots and spreader rows is shown in Figure 8.

4.5 DISCUSSION

This study confirmed the potential damage of rusts on wheat in South Africa. Under ideal conditions and the presence of a virulent pathotype and susceptible cultivar, these diseases can cause significant economic losses. Depending on the time, number of fungicide applications and the susceptibility of the cultivar, a yield increase of 21 to 43% can be achieved. In a recent study, yield losses caused by stem rust were reduced by 50% with the application of fungicides at the correct time (Loughman, Jayasena & Majewski, 2005). Previously, Pretorius (1983) showed that losses due to stem rust ranged from 7% to 35%, depending on the cultivar. With regard to wheat leaf rust, Boshoff, Pretorius & Van Niekerk (2002) showed that a combined seven leaf and flag leaf spray increased mean yield by 56%. Averaged over fungicides, one spray at the flag leaf stage increased yield by 50%. A similar yield gain of 56% was reported for wheat leaf rust control by Eversmeyer, Browder & Young (1975).

In the present study, the application of fungicides during the flag leaf growth stage resulted in a higher test weight in both trials. The effect of fungicide applications to quantify stem rust damage was not well illustrated in both experiments because of the occurrence of leaf rust. More than 92% of the losses in 2005 were due to leaf rust.

Significant differences were found among fungicides in their ability to control stem and leaf rust. McRae & Platt (1987) showed that AUDPC was more reliable than other measures to determine effective rates of fungicides. Low AUDPC values calculated for leaf and stem rust infections were observed with the application of tebuconazole which transpired into higher yield and test weights. In field studies Loughman *et al.* (2005) found that tebuconazole was more effective than flutriafol. In another study it was shown that chlorothalonil (a non-systemic fungicide) has a reduced efficacy and low eradicant activity as opposed to propiconazole (a systemic fungicide) (Mayfield, 1985). Mayfield (1985) also found a clear relationship between grain yield and disease severity by showing that prevention of a 1% increase in rust severity avoided a 2% loss in grain yield.

Different application methods did not differ statistically and more refined experiments are needed to study the effect of a canopy spray on rust infections at the stem base. Loughman *et al.* (2005) found that when a fungicide is sprayed on slightly infected plants there is a subsequent reduction in stem rust development, while on heavily infected plants poor control was achieved. Application of fungicides at late booting was profitable when stem rust was present at that stage.

The results of this study showed that stem and leaf rust could severely reduce the yield of susceptible cultivars in South Africa. Experimenting with fungicide rates and timing on different cultivars over several years and locations are necessary for developing accurate recommendations (Christ & Frank, 1989). More detailed research is needed to provide this information for stem rust control in South Africa.

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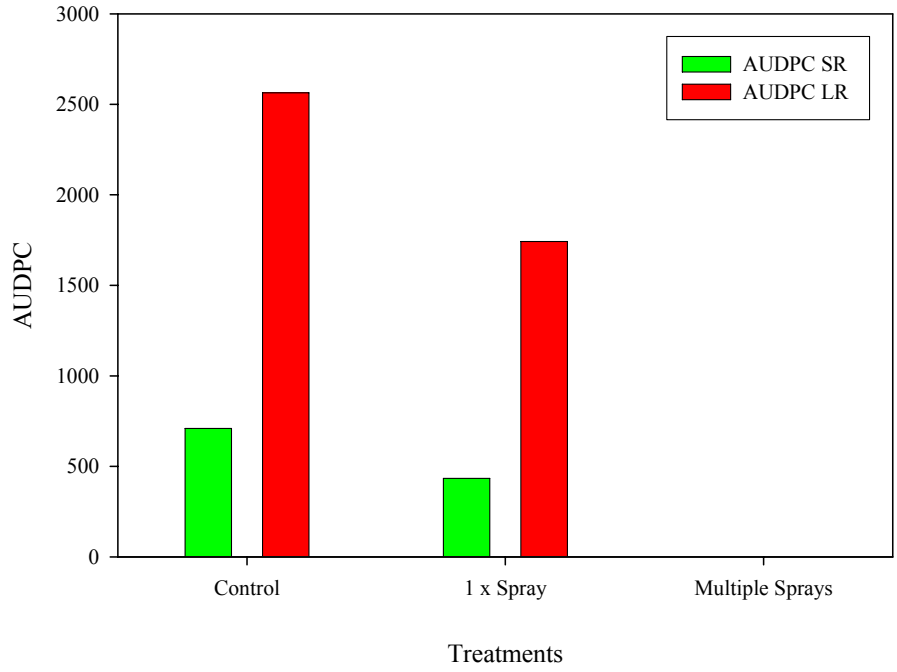

Figure 1. The mean area under the disease progress curve (AUDPC) calculated for each treatment in the 2005 experiment. The Tukey (P=0.05) values for comparing stem rust and leaf rust means are 36.08 and 40.03, respectively.

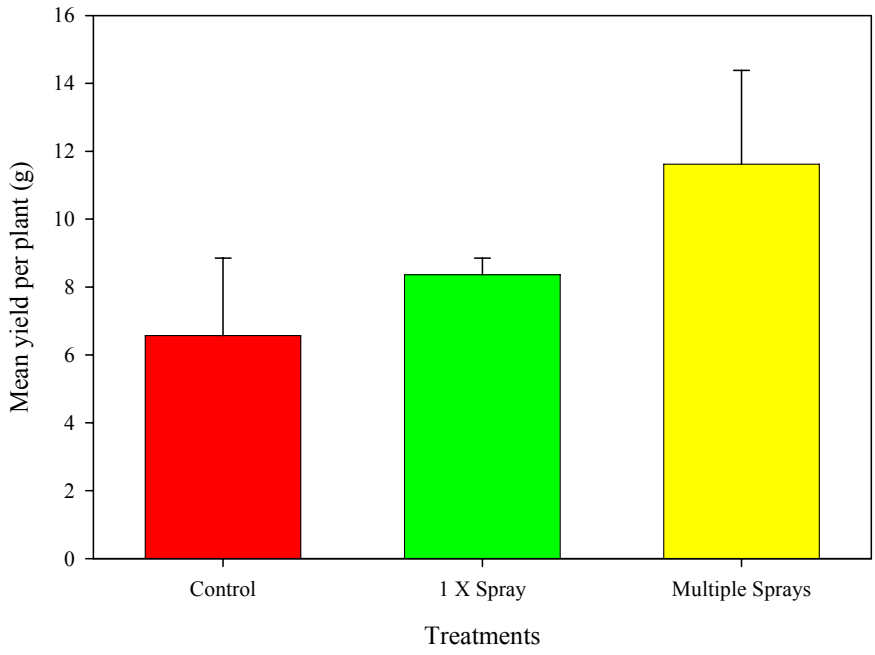

Figure 2. Mean yield for each treatment obtained over fungicides applied in 2005. Error bars indicate the positive standard deviation. The Tukey (P=0.05) value for comparing spray application means is 0.64.

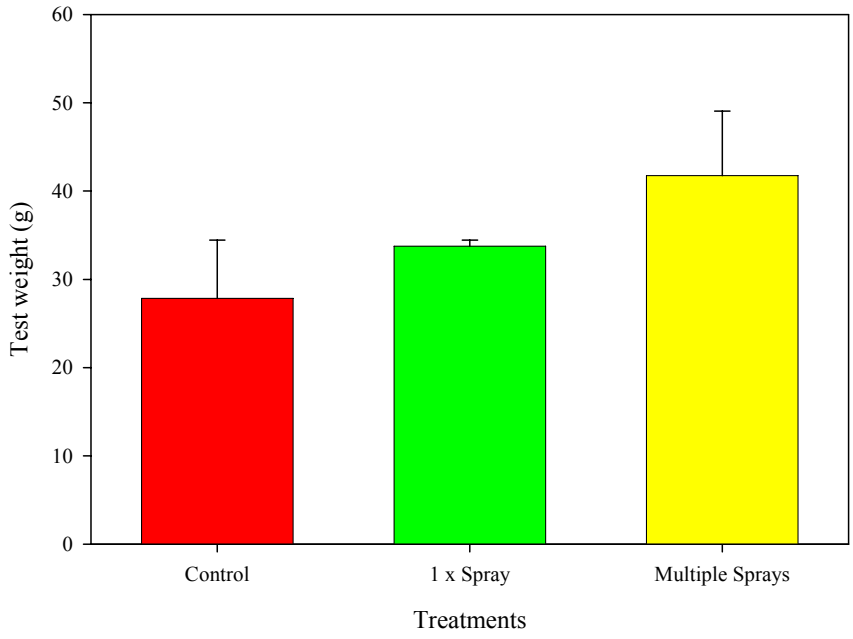

Figure 3. Mean test weight obtained over fungicides for each treatment in 2005. Error bars indicate standard deviation. The Tukey (P=0.05) value for comparing spray application means is 1.13.

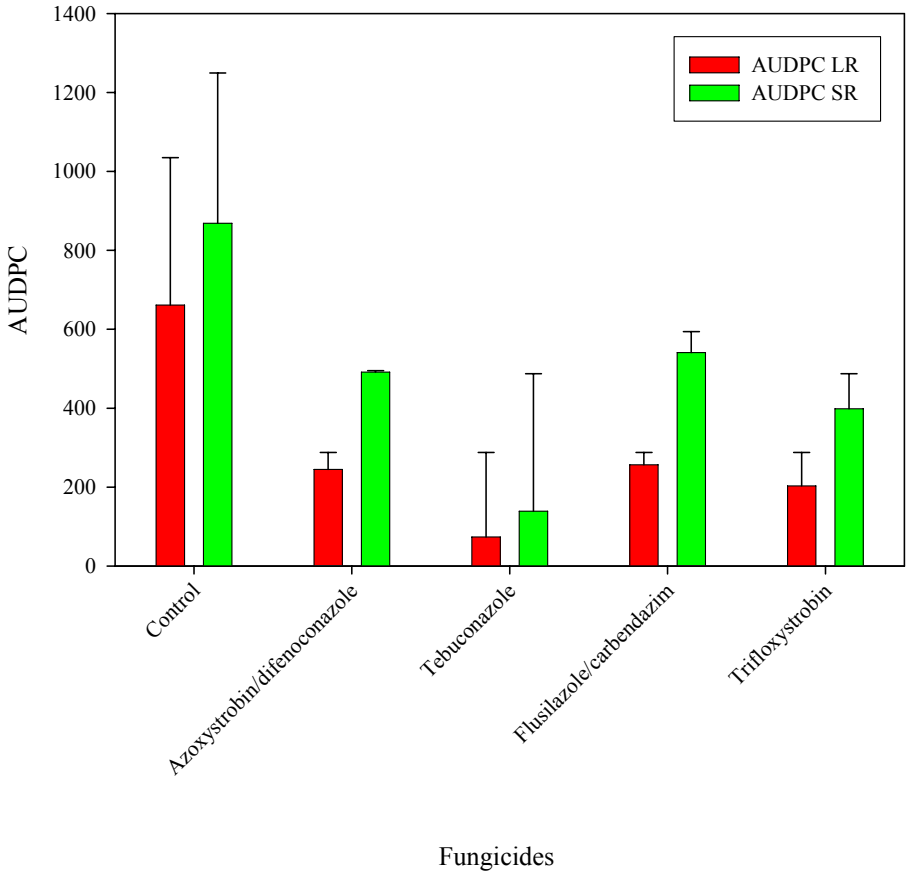

Figure 4. Mean area under disease progress curve (AUDPC) calculated for each fungicide applied over treatments in 2006. Error bars indicate positive standard deviations. The Tukey (P=0.05) values for comparing stem rust and leaf rust means are 178.27 and 99.71, respectively.

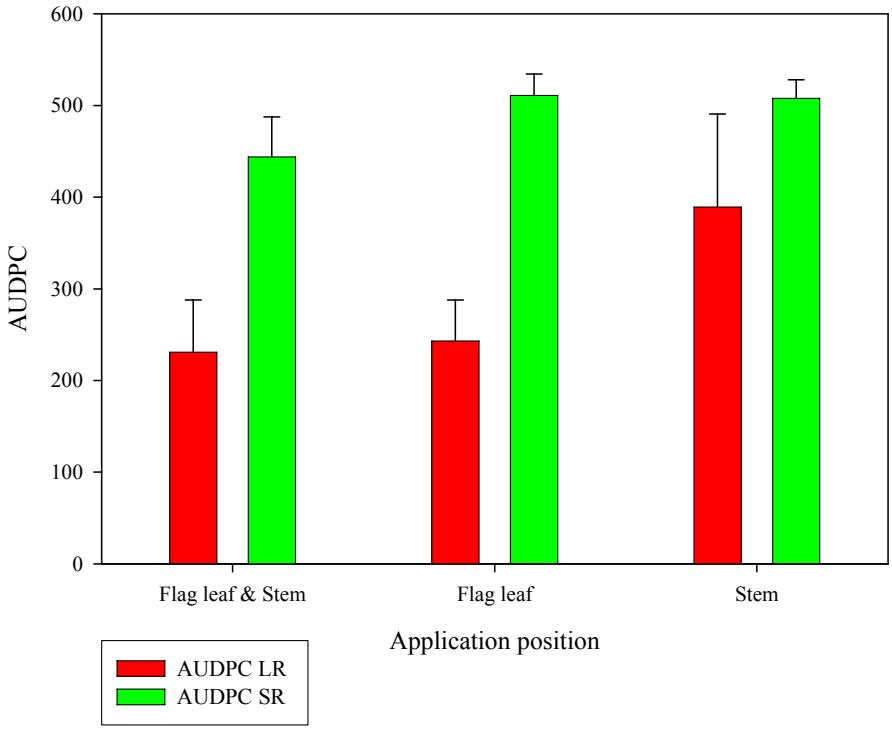

Figure 5. Mean area under disease progress curve (AUDPC) calculated for each application position over fungicides applied in 2006. AUDPC values for untreated stem and leaf rust plots were 868 and 661. Error bars indicate positive standard deviations. The Tukey (P=0.05) values for comparing stem rust and leaf rust means are 138.95 and 174.6, respectively.

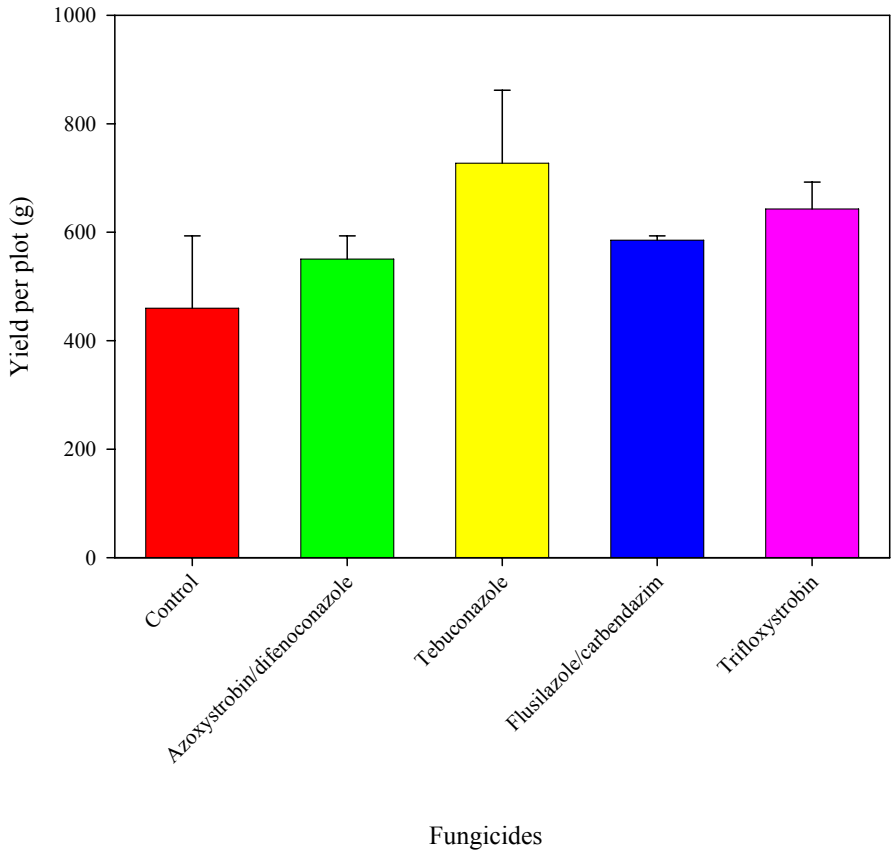

Figure 6. Mean yield obtained for the control and each fungicide treatment, averaged over application methods, in 2006. Error bars indicate positive standard deviations. The Tukey (P=0.05) value for comparing treatment means is 103.28.

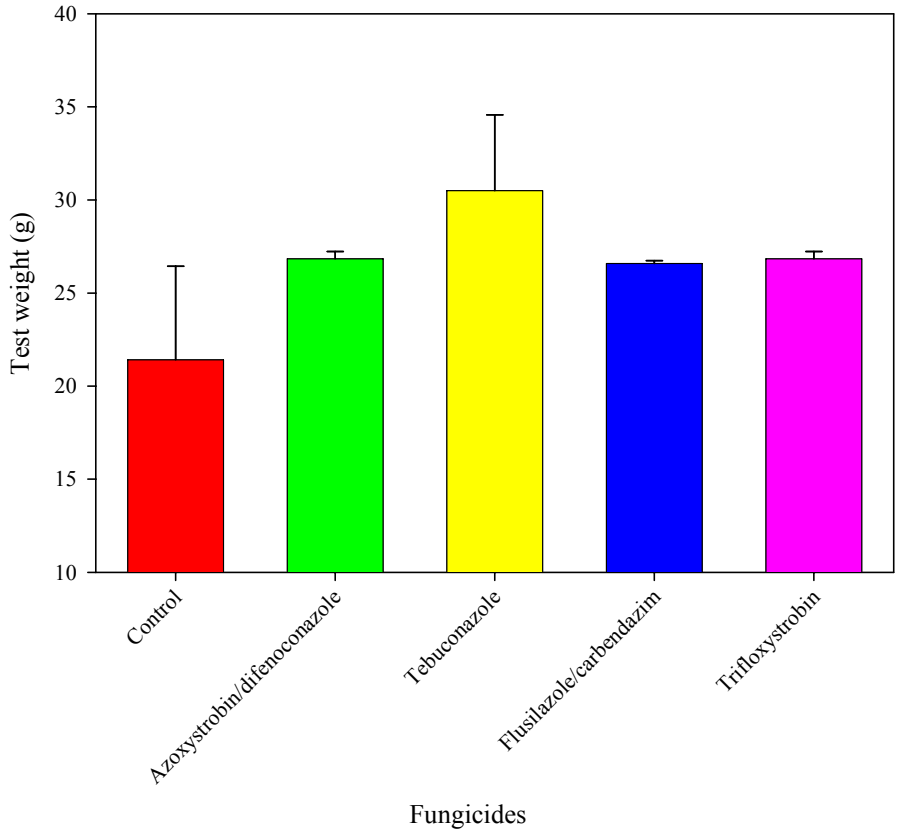

Figure 7. Mean test weight obtained for the control and each fungicide treatment, averaged over application technique, in 2006. Error bars indicate positive standard deviations. The Tukey (P=0.05) value for comparing treatment means is 5.41.

Figure 8. Wheat stem rust field trial at the Redgates Experimental farm, Greytown, KwaZulu-Natal in 2006. Rust spreader rows are indicated by solid arrows and fungicide-treated plots by broken arrows.

SUMMARY

Stem rust caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., is an economically important disease of bread wheat (*Triticum aestivum* L.) worldwide. The disease has often reached epidemic proportions in South Africa. The release of rust-susceptible cultivars in South Africa is of great concern and therefore research is needed to determine if chemical control is an economically viable method to control rust diseases. The recent increase of stem rust in the Western Cape production area of South Africa has led to a renewed interest in the use of fungicides.

In addition to chemical control, effective genetic control of rust diseases requires a coordinated effort, including pathotype monitoring, collection and characterization of sources of resistance, and resistance breeding. Data generated by pathotype surveys thus form an essential component of breeding programmes and are conducted by most wheat producing countries, principally to recognize pathogenic changes. The timely detection of stem rust pathotypes with new virulence is considered important to the South African wheat industry. A stem rust survey is conducted annually to monitor pathogenic variability in *Puccinia graminis* f. sp. *tritici* in the major bread wheat and triticale producing areas of South Africa. More than 70% of the isolates obtained from 2002 to 2004 were characterized as pathotype 2SA88, which unusual for local pathotypes, was virulent for *Sr8b* and *Sr38*. During the six year survey period, stem rust pathotypes 2SA4, 2SA55, 2SA88, 2SA99 and 2SA100 were detected on bread wheat and 2SA102, 2SA102K and 2SA103 on triticale.

To optimize *in vitro* testing conditions the effect of temperature on germination and germ tube growth of *P. graminis* f. sp. *tritici* as well as incubation periods were evaluated. Seventy eight percent of urediniospores had germinated on water agar in petri dishes after 6 h of incubation at 20 and 25°C. Low germination rates were observed at 30°C. Twenty-nine fungicides in nine chemical classes were evaluated *in vitro* for toxicity to stem rust urediniospores. Water agar petri dish plates were amended with fungicides at concentrations ranging from 10 to 0.0001 µl active ingredient / ml medium. Low EC_{50} (effective concentration that results in 50% inhibition) values were obtained with azoxystrobin, trifloxystrobin, kresoxim-methyl, mancozeb, azoxystrobin/ difenoconazole, iprodione, chlorothalonil and hexoconazole.

A shorter residual effect of trifloxystrobin occurred in wheat infected with stem rust in a glasshouse assay, whereas longer efficacy periods were determined for azoxystrobin/difenoconazole and tebuconazole.

Fungicide field trials were conducted at Greytown, South Africa to determine the effect of rust diseases on yield and test weight. Information on the efficacy of different foliar treatments in controlling these diseases was obtained. Although the emphasis was on stem rust, it was not possible to eliminate leaf rust from the field trials. In 2005, 92% of the yield reduction experienced was contributed by leaf rust infection whereas in 2006 32% of losses could be attributed to this disease. Stem rust on the other hand contributed 8% to losses in 2005 and 68% in 2006. Yield increase resulting from fungicide applications ranged from 36% to 45% over the two years. With a flag leaf treatment the mean yield increase per plant was 21% compared to 43% with a multiple-spray treatment in 2005. The highest yield increase was obtained with tebuconazole resulting in a 58% gain. Test weight increased by 29% with fungicide applications over the control plots. Further research is needed to optimize control by means of fungicides.

OPSOMMING

Stamroes, veroorsaak deur *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn., is ‘n ekonomies belangrike siekte op broodkoring (*Triticum aestivum* L.) wêreldwyd. Die siekte het gereeld epidemiese afmetings in Suid-Afrika aangeneem. Die vrystelling van vatbare kultivars is kommerwekkend en navorsing is nodig om te bepaal of chemiese beheer ‘n ekonomies-regverdigbare uitweg is om roessiektes te beheer. Die toename in stamroes in koringproduksiegebiede van die Wes-Kaap, Suid Afrika het gelei tot die algemene gebruik van swamdoders.

Bo en behalwe chemiese beheer benodig effekiewe genetiese beheer van roessiektes ‘n gekoördineerde poging insluitende patogeniese monitering, verkryging en karakterisering van weerstandsbronne en weerstandsteling. Data verkry vanaf patogeniese waarneming vorm ‘n essensiële komponent van ‘n teelprogram en word uitgevoer deur die meeste koringproduserende lande. Om stamroespatotipes betyds te identifiseer, veral ten opsigte van nuwe virulensie, is belangrik vir die Suid-Afrikaanse koringbedryf. Stamroesopnames word jaarliks uitgevoer om die patogeniese variasie van *P. graminis* f. sp. *tritici* in die belangrike koring en triticale produserende areas van Suid Afrika te moniteer. Meer as 70% van die isolate verkry gedurende 2002-2004 is gekarakteriseer as patotipe 2SA88, wat, ongewoon vir plaaslike patotipes, virulent is vir *Sr8b* en *Sr38*. Gedurende ‘n sesjaar periode is stamroespatotipes 2SA4, 2SA55, 2SA88, 2SA99 en 2SA100 geïdentifiseer op broodkoring terwyl patotipes 2SA102, 2SA102K en 2SA103 geïdentifiseer is op triticale.

Om *in vitro* toetse te optimiseer is die effek van temperatuur op ontkieming en kiembuislengte van *P. graminis* f. sp. *tritici*, sowel as inkubasieperiode geëvalueer. Agt en sewentig persent van urediniospore het ontkiem op wateragar petribakkies na 6 h van inkubasie by 20 en 25°C. Lae ontkiemingswaardes is verkry by 30°C. Nege-en-twintig swamdoders in nege chemiese groepe is *in vitro* getoets vir toksisiteit teenoor stamroesurediniospore. Swamdoders teen konsentrasies van 10 tot 0.0001 µl aktiewe bestanddeel per ml medium is by petribakkies met wateragar gevoeg. Lae EK_{50} waardes (effektiewe konsentrasie wat lei tot 50% inhibisie) is verkry met azoxystrobin, trifloxystrobin, kresoxim-methyl, mancozeb, azoxystrobin/ difenoconazole, iprodione, chlorothalonil en hexoconazole. ‘n Korter nawerking is

verkry met trifloxystrobin op koring geïnfekteer met stamroes in glashuistoetse, terwyl azoxystrobin/difenoconazole en tebuconazole langer nawerkingsperiodes getoon het.

Swamdoderveldproewe is uitgevoer by Greytown, Suid Afrika om die effek van roessiektes op opbrengs en 1000-korrel massa te bepaal. Inligting oor die effektiwiteit van verskillende blaarbespuitings in die beheer van die siektes is verkry. Al was die primere fokus gerig op stamroes, kon daar nie verhoed word dat blaarroes die proewe infekteer nie. In 2005 is 92%, en in 2006, 32% van die opbrengsverliese veroorsaak deur blaarroesinfeksies. Stamroes het bygedra tot 8% van verliese in 2005 en 68% in 2006. Opbrengsverhoging verkry deur swamdodertoediening het gewissel van 36% tot 45% oor die tweejaarperiode. In 2005 is die gemiddelde opbrengs per plant verhoog met 21% deur ‘n vlagblaarbespuiting, vergeleke met 43% met herhaalde bespuitings. Die hoogste opbrengs verhoging is verkry met tebuconazole (58%). Duisendkorrelmassa het verhoog met 29% vergeleke met die kontrole. Verdere navorsing is nodig om chemiese beheer te optimaliseer.