A COMPARISON OF SCREENING TECHNIQUES FOR FUSARIUM HEAD BLIGHT OF WHEAT IN SOUTH AFRICA

by

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Submitted in fulfilment of the requirements for the degree

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in Plant Pathology

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NOVEMBER 2009

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C I P de Villiers

ACKNOWLEDGEMENTS

I hereby wish to express my sincere thanks to the following people:

1. Ms W-M Kriel and Prof Z A Pretorius for the support and guidance in preparation of the manuscript.

2. My husband Pierre, who always believed in me and encouraged me to continue with this study.

3. My mother who gave me support and encouragement during this study.

4. The Agricultural Research Council, Small Grain Institute (ARC-SGI), who provided me with financial support and the facilities to execute the trials in the greenhouse and field.

5. Our librarian, Juliëtte Kilian, for her invaluable support and Dr André Malan for guidance.

6. Nyiko Baloyi and Isaiah Moloi who assisted me in the greenhouse and field trials.

PREFACE

In South Africa wheat is planted in different production areas where diseases often are important production constraints. One of the most important diseases is Fusarium head blight which occurs mainly under centre pivot irrigation. This disease was first observed in 1980, but was officially reported in the late 1980's. From literature it is clear that the predominant *Fusarium* species that cause head blight worldwide are *F. graminearum*, *F. culmorum* and *F. avenaceum.* The causal species in a region depends on the climate, crop rotation and the amount of inoculum present.

Typical symptoms include the appearance of a watery soaked lesion in one or more spikelets of a healthy looking wheat head, showing the point of entry. As the infection spreads, the upper and lower part of the head (adjacent to initially infected spikelet) will also become blighted. Prematurely blighted heads appear after flowering in contrast to healthy heads which remain green. Mycelial growth may also be visible after infection. Most infections reduce the amount of seed, their mass and grain quality. Infected seeds are normally blown out by the combine because of their low seed weight. If infected seed is planted in the following year, it may result in seedling blight. *Fusarium* does not occur annually and breeding programmes based on natural infection are less successful. The control options for Fusarium head blight in South Africa are limited since no fungicides have been registered against this disease. Environmental conditions and tillage practices also have an influence on disease incidence. It is important to reduce the amount of inoculum by removing stubble from the field. In South Africa there is currently no cultivar that is resistant against head blight and breeding programmes rely on field and greenhouse screenings for quantification of cultivar reactions.

From literature there are a number of methods to screen for resistance in germplasm using different inoculation techniques. These techniques include single floret inoculation, spray inoculation and the distribution of infected grain. All of these methods can be used in the field or greenhouse.

Two objectives were identified for this study. The first objective was to determine the virulence of *Fusarium graminearum* isolates from the Prieska region in the greenhouse and to identify a solid medium for vigorous sporulation in the laboratory. Various attempts have been made to improve media for *Fusarium* species, some of which will be investigated here. The second objective was to identify and confirm inoculation techniques for Type I and Type II resistance in the greenhouse and field.

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

Fusarium infected heads in a wheat field near Winterton, KwaZulu-Natal.

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CHAPTER 1

A REVIEW OF FUSARIUM HEAD BLIGHT ON WHEAT

1.1 INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple food crops in South Africa and its origin can be traced back as far as 1652 when it was planted for the first time by Dutch settlers in the Cape. Wheat production was so well established that on rare occasions it was exported to India during 1684 (Van Niekerk, 2001). Currently, wheat is produced in all nine provinces namely the Western-, Eastern- and Northern Cape, Free State, KwaZulu-Natal, Limpopo, Mpumalanga, Gauteng and North West. The production areas of wheat depend on the soil type, soil depth, soil water content, the environment and the availability of irrigation (Van Niekerk, 2001).

South African wheat producers planted 748 000 ha during the 2008 season, with a total production of 2 031 000 tons. The total income value is more than R4.468 billion. During this season 223 000 tons of wheat were exported with the local consumption estimated as 2 844 000 tons (Anonymous, 2009).

One of the major limiting factors in wheat production in South Africa is diseases, decreasing the yield, quality and profitability for producers. Fusarium head blight (FHB) is one of the most important diseases occurring on wheat under irrigation (Scott *et al*., 1988).

FHB, also known as ear blight, scab, white heads and pink mold, is mainly caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schw.) Petch). Since the disease was initially recorded on wheat, barley and other small grains, 17 different *Fusarium* species have been associated with the disease. *Fusarium graminearum* is the species that predominates internationally, followed by *F. culmorum* (W.G. Smith) Saccardo and *F. avenaceum* (Fries) Saccardo (*G. avenacea* Cook) (Parry *et al*., 1995; Ruckenbauer *et al*., 2001).

FHB is an important disease throughout the wheat growing areas of the world. The disease is more severe when wheat is sown in the residue from a previous host crop such as maize (*Zea mays* L.), followed by warm humid conditions during flowering. FHB is currently one of the most devastating

diseases on wheat and barley (*Hordeum vulgare* L.) internationally (Cook, 1981; McMullen *et al*., 1997; Steffenson, 2003). *Fusarium graminearum* causes head blight, stalk and ear rot of maize and may cause root rot in cereals (McMullen *et al*., 1997; Beyer *et al*., 2004). Most *Fusarium* fungi are soil-inhabiting and may grow on living plant material as well as on dead organic material (as facultative saprophytes) (Pomeranz *et al.,* 1990). Residues of the previous cereal crop produce inoculum in the form of ascospores and conidia. Airborne ascospores have been linked to factors such as rainfall and humidity (Markell & Francl, 2003).

In South Africa, FHB was observed for the first time in the 1980's on irrigated wheat in the North West Province, but only reported in 1988 from the Prieska area (Scott *et al*., 1988). A map showing the wheat irrigation areas in South Africa can be seen in Fig. 1.1. Crown rot of wheat caused by *F. pseudograminearum* Aoki & O'Donnell was reported in late 1980 on irrigation wheat (Marasas *et al*., 1988).

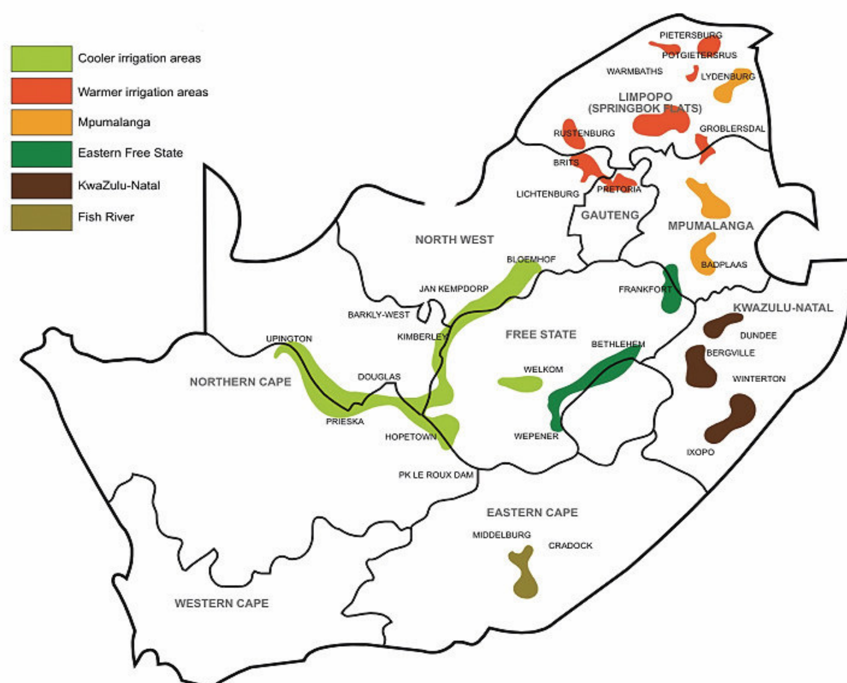

Figure 1.1: Irrigation areas in South Africa where wheat is produced (Anonymous, 2001).

Internationally, there are more reports of FHB on wheat than on barley, largely due to the economic importance of wheat over barley. Although the

same organism attacks wheat and barley, their reaction to the organism differs (Steffenson, 2003). In general, barley is more resistant to *Fusarium* since the spikelets are enclosed in the flag leaf sheath during flowering, probably reducing the chances of infection (Steffenson, 2003).

FHB is recognized by the appearance of one or more prematurely blighted spikelets after flowering. The infected ears develop to a bleached straw colour, whereas healthy plants remain green (*Pomeranz et al.,*1990; Stack, 2000). Peach to pink fungal mycelium and conidia form on infected kernels (Steffenson, 2003). Most infections result in reduced kernel number, kernel weight, grain quality and spikelet sterility, causing losses of up to 100% (Schroeder & Christensen, 1963; Snijders, 1990; Bai & Shaner, 1994; McMullen *et al*., 1997; Nicholson *et al*., 2007). In contrast, infections that occur during the late stages of kernel development will have little impact on yield and yield losses (Steffenson, 2003).

The diseased grain may also contain mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS) or zearalenone (ZEA), all potentially hazardous to humans and animals (McMullen *et al*., 1997; Steffenson, 2003).

FHB does not necessarily occur in consecutive years therefore a breeding programme based on natural infection is not viable (Steffenson, 2003). If inoculum is constantly present, the outbreak of an epidemic may occur, but outbreaks also depend on favourable climatic conditions (Steffenson, 2003).

The aim of this literature review is to summarize the current literature on FHB of wheat, with emphasis on different inoculation and screening techniques used in the greenhouse and field.

1.2 CAUSAL ORGANISMS

Since the earliest disease records, FHB has been reported in most wheat growing areas in the world (Leslie & Summerell, 2006). At least 17 different *Fusarium* species have been associated with this disease (Parry *et al*., 1995).

1.2.1 Taxonomic criteria

During the past 100 years the taxonomy of the genus *Fusarium* has undergone a number of changes and the concept of a species within a genus

has varied greatly. According to Leslie and Summerell (2006), the basis for all modern taxonomic systems was the work of Wollenweber and Reinking when they created a generic system based on 16 sections, 65 species and 77 sub-specific varieties and forms. Most of these species described by Wollenweber and Reinking are still in common use today (Pomeranz *et al.,* 1990; Leslie & Summerell, 2006).

During the 1940's and 1950's Snyder and Hansen reduced the number of species within the genus to nine. They demonstrated the use of cultures derived from single spores for reliable identification. The Snyder and Hansen species concepts were popular because they were easy to apply and virtually every isolate could be identified to species with ease. However, the taxa used were polyphyletic and the data generated during this time are difficult to interpret (Leslie & Summerell, 2006).

In the 1970's Booth (1971) published *The Genus Fusarium*. He included keys to the sections and species of *Fusarium* in a taxonomic system that borrowed heavily from Wollenweber and Reinkings' approach. Booth introduced the use of the morphology of the conidiogenous cells, as a species-level diagnostic character. Today conidiogenous cell morphology is essential for distinguishing some of the species in sections *Liseola* and *Sporotrichiella* (Leslie & Summerell, 2006).

The species that cause the disease in one region, depends on the climate and the amount of inoculum present (Tekauz *et al*., 2000). This may therefore vary within regions as well as between regions (Gale, 2003). Occurrence of *Fusarium* species in cooler parts includes species such as *F. culmorum, Microdochium nivalis* (Fr.) Samuels & I.C. Hallet and *Gibberella avenacea* (Scott *et al*., 1988). *Fusarium graminearum* occurs in hot and humid climates especially during anthesis, whereas *F. pseudograminearum* is more common in the drier areas (Shaner, 2003).

In culture, *Fusarium* species often produce mycelium shades of pink, white or yellow. Two types of conidia are present namely microconia and macroconidia. Microconidia are oval and one-celled and they are not always present. Macroconidia are often distinctly fusoid and septate (Wiese, 1987).

1.2.2 Pathogenicity

Variation is one of the outstanding characteristics of *Fusarium graminearum* because of the differences in the virulence of different isolates to maize and other small grains. Differences in pathogenicity among isolates are well known, but evidence of interactions between cultivars and pathogen isolates is yet to be identified. Different isolates or species may predominate in different years on account of weather conditions (Dill-Macky, 2003). Consequently, isolates observed from maize and crowns of wheat may produce severe head blight, but crown rot is produced only by isolates from infected crowns. *F. graminearum* has been differentiated into two naturally occurring forms that are referred to as Group I and Group II. Group I contains the *F. graminearum* isolates normally associated with diseases of crowns of plants, is heterothallic and poorly fertile or infertile (Dill-Macky, 2003). This group does not form perithecia in culture and rarely form perithecia in nature. Group II contains the *F. graminearum* isolates normally associated with aerial diseases of plants, is homothallic, readily forms perithecia in monoconidial cultures and can produce the *Gibberella* stage in nature (Parry *et al.*, 1995).

Group II is the most common form of the two and it is widely accepted that *F. graminearum* Group II is the cause of FHB (Pomeranz *et al*., 1990; Parry *et al*., 1995). Characteristics such as colony morphology, growth and conidial dimensions, as well as the intensity of sporulation can be used to distinguish Group I and Group II, but perithecia formation is the most important criterion to distinguish Groups I and II (Dill-Macky, 2003).

Group I has since been re-classified because these different fungal species produce their own characteristic sexual state as can be seen in Table 1.1.

Table 1.1: The differences between the FHB pathogen and the crown rot pathogen in the reclassification of Group I (Liddell, 2003).

Description	FHB pathogen	Crown rot pathogen
Old name	*F. graminearum* Group II	*F. graminearum* Group I
New name	*F. graminearum*	*F. pseudograminearum*
Sexual state	*Gibberella zeae*	*Gibberella coronicola*

The groups were identified as genetically and biologically distinct populations and there is evidence that *G. coronicola* Aoki & O'Donnell is an

important cause of FHB (Liddell, 2003; Mitter *et al*., 2006; Xie *et al*., 2006). In contrast with earlier findings, Mitter *et al.* (2006) found that many of the *Fusarium* species that cause FHB, can also cause crown rot. These species include *F. culmorum, F. aveaceum, F. graminearum* and *F. acuminatum* Ellis & Everhart. Evidence shows that the pathogen biology and epidemiology of FHB and crown rot are linked.

1.3 ETIOLOGY

Understanding the life cycle of FHB is important for its relation to seedling blight as well as head blight on small grains. The initial source of *Fusarium* inoculum is from the crop residue and may consist of ascospores, hyphal fragments, macroconidia or chlamydospores (Sturz & Johnston, 1985; Parry *et al*., 1995). Sowing cereal seed into *Fusarium* infested soil may result in the infection of plants and the development of seedling rot. *Fusarium-* infected grain resulting from the development of FHB can, if used as seed, provide an important source of inoculum for the development of seedling blight that will complete the disease cycle (Dill-Macky, 2003). In nature, hyphal fragments are an important source of inoculum for root infections. Later in the growing season air-borne inoculum may infect the heads of plants. A complete disease cycle of the *Fusarium* fungus can be seen in Fig. 1.2. Disease severity varies between years, since FHB development depends on favourable environmental conditions from flowering through kernel development (McMullen & Stack, 1999).

Macroconidia are disseminated by rain splash over short distances and are ideal propagules for use as inoculum (Bai & Shaner, 1994).

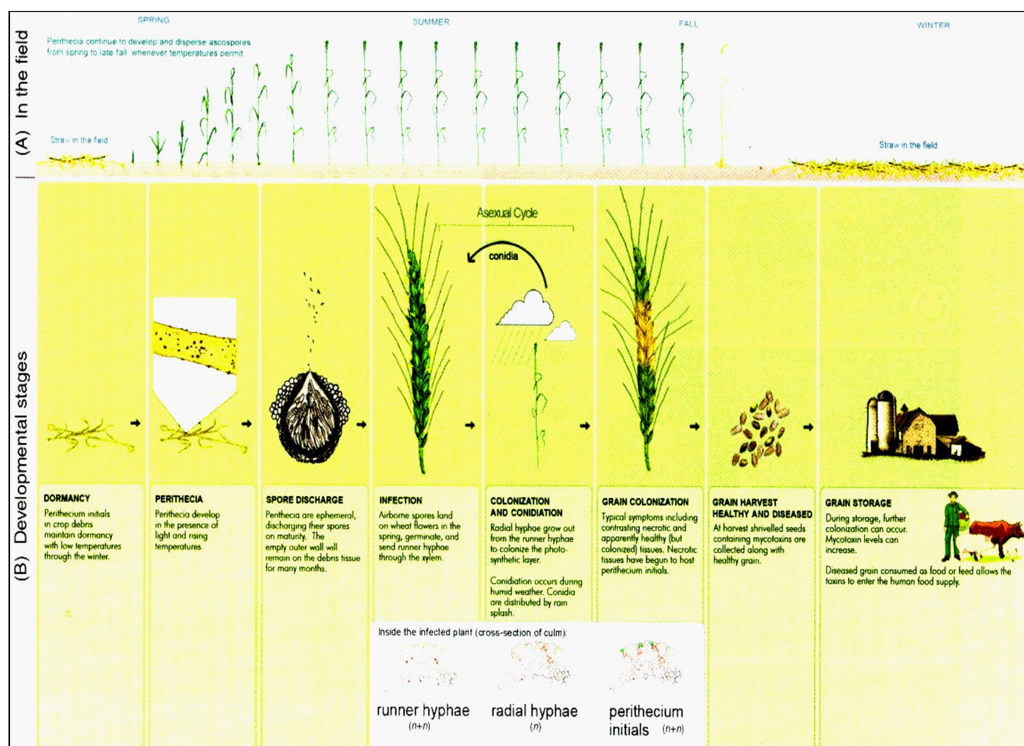

Figure 1.2: The disease cycle of *Fusarium* in small grains (Trail *et al.*, 2005).

Macroconidia and hyphal fragments are used for the production of inoculum for screening purposes.

1.3.1 Sources of inoculum

The primary sources of inoculum include crop residues on and in the soil, infected plants and infected seed. Crop debris, including old stalks and debris of maize, stubble of wheat, barley and other cereals, ensures high inoculum levels in the form of conidia and ascospores (Atanasoff, 1920; Sutton, 1982; Scott *et al.*, 1988). The amount of inoculum is directly correlated to the density of the crop residue on the soil surface as well as the duration that the crop debris, such as maize, wheat or barley stubble, remains on the soil after harvesting (Sutton, 1982; Windels *et al.,* 1988; McMullen *et al*., 1997; Shaner, 2003).

The saprophytic survival of the pathogen in residues could provide inoculum from one season to the next (Boshoff, 1996). Conidia can be

dispersed to wheat florets and stems from crop residues by water splash to produce head blight or stem rot. Some species of *Fusarium* can survive for long periods in soil (Shaner, 2003). Wind and insects also aid in the transfer of the inoculum from one plant to another (Atanasoff, 1920). Retained seed, infected with *Fusarium*, can reduce seed germination and increase the incidence of root rot and seedling blight (Steffenson, 2003).

1.3.2 Inoculum production and dispersal

In the case of *G. zeae,* ascospores represent an important source of inoculum (Parry *et al.*, 1995). Warm and moist conditions are needed for the formation of both the ascospores (sexual spores) and macroconidia (asexual spores). Macroconidia of *F. graminearum* (Fig. 1.3) are between 3.5 – 4 x 40 – 80 µm and three- to seven septate with a well-marked foot cell (Cappellini & Peterson, 1965; Booth, 1971; Sutton, 1982; Beyer *et al.*, 2004).

Inoculum consists of ascospores produced within perithecia, macroconida produced on sporodochia, chlamydospores surviving on the soil surface or on crop debris and hyphal fragments that survive in mainly maize residues. Both ascospores and macroconidia can be the primary inoculum source because the fungus needs to reach its infection site through aerial dispersal (Sutton, 1982).

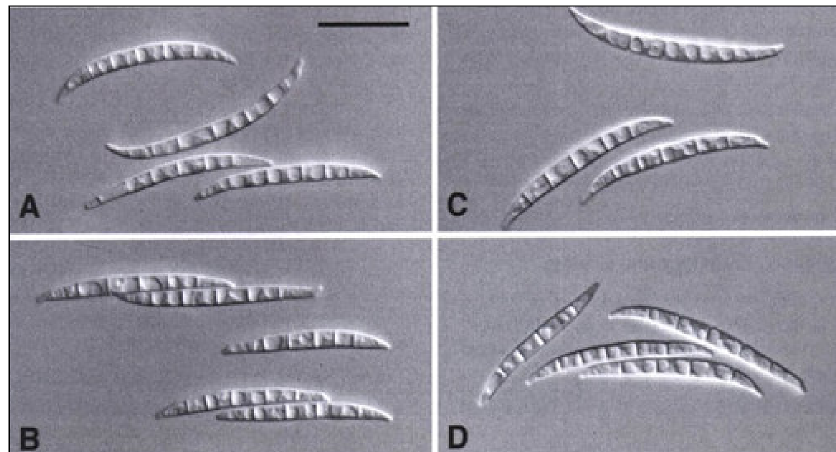

Figure 1.3: A - D: Macroconidia of *Fusarium graminearum* are between 3.5 – 4 x 40 – 80 µm and the three- to seven-septate with a well-marked foot cell. Scale bar is 25 µm (Leslie & Summerell, 2006).

Ascospores produced from purple to black perithecia on its host, are distinctive and uniform in shape, septation and size, and can be recognized with ease. On the other hand, macroconidia are highly variable and not readily distinguished from other fusaria unless they are cultured (Sutton, 1982).

Favourable temperatures for the production of ascospores and macroconidia vary between 16℃ to 36℃. Temperatures for the formation of perithecia vary between 16℃ and 31℃, but the optimal reported temperature for perithecium formation is 29℃. Ultraviolet light shorter than 390 nm is needed for perithecial initiation and they will mature between nine to ten days under favourable conditions (Sutton, 1982). Perithecia develop on seedlings, infected kernels, on residue and heads of various cereal crops (Atanasoff, 1920; Wiese, 1987).

Moist conditions that include high relative humidity of more than 92%, rainfall and/or irrigation, are required for the production of ascospores and macroconidia. Ascospore release coinciding with high humidity is needed during anthesis to produce FHB (Trail *et al.*, 2002; Markell & Francl, 2003). When ascospore release does not correspond with anthesis of a cereal crop, infections are reduced (Nelson *et al.*, 1981).

Ascospores are disseminated mainly at night and wind also plays a role in the spreading of inoculum. Research has shown FHB to be less common in areas that are sheltered from wind because wind is one of the primary transport mechanisms (Atanasoff, 1920; Sutton, 1982). Cool to moderate temperatures (13℃ – 22℃), favour inoculum dispersal, accompanied by RH levels between 95% – 100% (Nelson *et al.*, 1981; Sutton, 1982; Shaner, 2003).

According to Parry *et al.* (1995) rain and dew play an important role in the initial release of ascospores, but dry periods are needed for the forceful discharge of ascospores in the air. Rain and irrigation are important in the dispersal of *Fusarium* inoculum with up to 89% of heads infected by *F. avenaceum, F. culmorum, F. graminearum* or *F. poae* in wheat crops under overhead irrigation (Strausbaugh & Maloy, 1986; Murray *et al.*, 2009).

Birds, insects and other arthropods also serve as vectors of *F. graminearum* in the field. *F. graminearum* was collected from adult sap-feeding picnic beetles (*Glischrochilus quadrisignatus*) in maize and maize root worm beatles (*Diabrotica longicornis*) (Munkvold, 2003). Birds such as starlings shred the husks, puncture the kernels and remove the contents of the pericarp on maize (Gordon, 1959; Sutton, 1982). Splashing or wind-driven rain is widely

regarded as the main dispersal mechanism for macroconidia of *F. graminearum* (Sutton, 1982). Barley thrips, mites and grasshoppers are also vectors for *F. graminearum* (Sturz & Johnston, 1985; Parry *et al.*, 1995; Kemp *et al.*, 1996).

1.3.3 Infection and colonisation

Several factors determine disease development once *Fusarium* inoculum has spread to the heads. These include the susceptibility of cereal spikes to *Fusarium* infection and weather conditions. Infection usually takes place from anthesis to the soft dough stage (Parry *et al.*, 1995).

During the latter part of anthesis, cereals are more susceptible to *Fusarium* infection (Atanasoff, 1920) but the stage of peak receptivity differs among cultivars (Schroeder & Christensen, 1963). Increased infection of cereal spikes by *Fusarium* species during anthesis, has been documented during warm and wet weather conditions (Parry *et al.*, 1995). Ascospores are carried by air currents and deposited on or inside one or more spikelets. During humid weather the spores will germinate and initiate primary infection. These spores can germinate fairly effectively within 3 h at 28°C (Shaner, 2003). Fungal spores may land on the exposed anthers of the flower and then grow into the kernels, glumes and/or other head parts (McMullen & Stack, 1999). The incubation period (days from inoculation until appearance of symptoms) is influenced by weather, temperature and moisture period (Shaner, 2003). Where temperatures range between 25°C and 30°C, with continuous moisture, symptoms may develop within three days after infection (Wiese, 1987). The parenchyma of the pericarp begins to break down after infection, the cytoplasm and nuclei of the cells disappear and the cell walls break down. The fungus then colonizes both the inside as well as the outside of the kernel (Parry *et al.*, 1995; Nicholson *et al.*, 2007).

1.3.4 Symptoms

Typical symptoms of infection by *Fusarium* show a brown, water soaked lesion on the spikelet and a loss of chlorophyll in the spikelets and rachis (Pugh *et al.*, 1933; Parry *et al.*, 1995; McMullen & Stack, 1999; Nicholson *et al.*, 2007). This discolouration spreads in all directions in the head. During epidemics, disease severity increases with time. Pugh *et al.* (1933) found that the

progression of infection followed the general progression of anthesis, beginning in the centre of the head and proceeding outward towards the tip and the base of the head. Symptoms will also spread throughout the rachis both apically and basally from the point of infection. Aerial mycelium spreads externally from originally infected spikelets to adjacent spikelets during optimum weather conditions (Wiese, 1987). If weather is wet and warm, a salmon-pink to orange fungal growth will be evident on the affected heads and along the edge of the glumes or the base of the spikelet (Wiese, 1987; Parry *et al.*, 1995).

Dark brown discolouration of the rachis and the stem tissue coincides with the clogging of vascular tissue and subsequent prevention of translocation of water and nutrients, which can cause heads to ripen prematurely (Schroeder & Christensen, 1963; Bai & Shaner, 1994; Nicholson *et al.*, 2007). Figure 1.4 shows the distinct discolouration of wheat heads infected with *F. graminearum*.

Figure 1.4: Distinct symptoms of infection with *Fusarium graminearum* (from greenhouse trials). Note the water soaked lesion in the middle of the head and subsequent spread of the pathogen to adjacent spikelets.

Kernels infected with *Fusarium* are usually tan, tan-orange, brown or dark brown in colour and thin or flattened due to the shortage of water and nutrients (McMullen & Stack, 1999; Tekauz *et al*., 2000). Diseased and healthy wheat kernels can be seen in Fig. 1.5.

Figure 1.5: Visually scabby wheat kernels infected with *Fusarium graminearum* on the right and healthy wheat kernels on the left.

Advanced infections show black perithecia on the surface of kernels (Mathre, 1997; Tekauz *et al*., 2000; Shaner, 2003; Steffenson, 2003). FHB is easy to recognize in the field because no other disease produces quite the same symptoms and white heads are distinctive in a green field (Mathre, 1997). Retained seed infected with *Fusarium* could reduce seed germination and increase the incidence of root rot and seedling blight (Steffenson, 2003; Nicholson *et al*., 2007). Infected kernels may contain mycotoxins which are hazardous to humans as well as animals (Ruckenbauer *et al*., 2001).

1.3.5 Mycotoxin production

Fusarium mycotoxins have been studied for their involvement in pathogenicity of the fungus during infection. *F. graminearum* and other *Fusarium* species have the potential to produce secondary metabolites in culture including trichothecenes, culmorins, fusarins and fumonisins as well as a variety of other compounds such as moniliformin and zearalenone (Murray, *et al*., 2009).

It is known that mycotoxins can cause health problems such as mycoses when fed to farm animals such as swine. Feed refusal, vomiting and hyperestrogenism are some of the symptoms exhibited by swine (McMullen *et*

al., 1997; Steffenson, 2003). Mycotoxins may also influence the reproductive performance in some livestock (Schwarz, 2003).

The major focus on food safety around the world has concentrated on the trichothecenes and zearalenone (ZEA). The trichothecenes include deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-DON), diacetoxyscirpenol (DAS), nivalenol (NIV), 4-acetylnivalenol (4-ANIV), 3-acetildeoxynivalenol (3-DON), and 4,15-diacetylnivalenol (4,15-Daniv). The most important mycotoxin, in terms of human exposure, is DON. The trichothecene NIV produced by some isolates of *Fusarium* is believed to be more toxic than DON and hence should be of more importance with respect to food safety (Nicholson *et al.*, 2007).

Grain containing one or some of these mycotoxins, may be downgraded or rejected by the food and brewing industries because of the health risks associated with mycotoxins. Although barley is used as a staple food in some regions, it is also used for the production of malt and beer. When *Fusarium* infected grain is used to produce beer, problems such as gushing of beer (uncontrolled foaming) are experienced. Mycotoxicosis symptoms in humans include nausea, diarrhoea, abdominal pain, dizziness and fever (Parry *et al*., 1995; Schwarz, 2003; Steffenson, 2003; Nicholson *et al.*, 2007). Zearalenone is always more abundant in maize than in wheat and barley (Mirocha, 2003).

1.3.6 Economic importance

FHB is an important disease of wheat and other small grains worldwide (Cook, 1981; McMullen *et al*., 1997). Infections might lead to a reduction in yield, grain and seed quality. Contaminated grain is a source of inoculum for seedling blight and foot rot. *Fusarium graminearum* affects the quality of the grain, leading to reduced seed germination and vigour (Parry *et al.*, 1995). If the grain contains mycotoxins such as DON, it poses health risks and diminishes the value of the affected grain (McMullen *et al.*, 1997; Windels, 2000).

In Paraguay the weather conditions in 1972 – 1975 favoured FHB epidemics and accounted for losses up to 70%. Although FHB has been recorded in North America for more than 100 years it only recently emerged as a more chronic problem. In the United States of America (U.S.A.) and Australia *F. graminearum* is the dominant species causing FHB. Damage due to FHB in the

U.S.A. was estimated at more than one billion dollar in 1993 and $500 million in 1994 (Windels, 2000).

Epidemics in China are most common and severe in the Yangtze River Valley and affect more than 7 million ha of wheat. In epidemic years it is estimated that China might lose up to 2.5 million tons of grain due to FHB infection (Windels, 2000). Important factors contributing to the increased frequency of FHB epidemics include shorter crop rotations and reduced tillage in recent years (Dill-Macky & Jones, 2000). Land planted to wheat in Minnesota decreased by 31% due to *Fusarium* epidemics between 1992 and 1998 (Nicholson *et al.,* 2007).

In South Africa double cropping with maize and wheat, together with reduced tillage and the cultivation of susceptible cultivars, are the main factors that contribute to FHB epidemics under irrigation. Understanding the pathogen population and an increase in resistance levels of commercial cultivars can contribute to the management of FHB in the future (Kriel & Pretorius, 2008).

1.4 DISEASE CONTROL

Certain measures must be considered to reduce the quantity and dispersal of inoculum or the prevention of infection, should inoculum be present. These measures include crop rotation, land preparation and weed control (Parry *et al.*, 1995). To achieve these goals the following needs to be considered:

1.4.1 Cultural management

In 1891, Arthur suggested the first management practice for FHB control. He suggested that cereal must be planted earlier in the planting season to escape infection of *Fusarium* during flowering (Cook, 1981).

Ploughing and/or burning might significantly reduce the amount of *Fusarium* inoculum in the field. Unfortunately, ploughing may also cause erosion, loss of soil moisture and it is costly and time consuming (Steffenson, 2003). Since the burning of crop debris has been banned in the European Community, mouldboard ploughing is now the only option for the disposal of crop debris (Parry *et al.*, 1995).

Tillage practices can bury stubble that may be the source of inoculum (McMullen & Stack, 1999). Where reduced tillage practices are used, maize

residue is still abundant during the second spring after harvest of the maize crop, because maize residue lasts much longer than residue of small grains (Shaner, 2003).

Maize debris is one of the main sources of inoculum (McMullen & Stack, 1999). Abundance of inoculum depends on how long the residue remains intact after harvest of the crop and how well the fungi survive in this residue (Shaner, 2003). To control FHB, early ploughing of cereal stubble and volunteer plants should be carried out wherever possible, since perithecia can only release inoculum from infested residue that is retained on the soil surface (Cook, 1981; Jones & Clifford, 1983). Rotations of three years between crops of maize or small grains would be sensible in the case of reduced tillage, since studies showed that sporulation of the fungus is reduced within a three year period. This will provide a measure of FHB control (Shaner, 2003).

Atanasoff (1920) and Jenkinson & Parry (1994) recognized the importance of weed control, especially annual broad-leaved weeds, as they suggested that a rise in FHB incidence is evident with increasing weed populations. Grass weeds such as paradoxa grass (*Phalaris paradoxa* L.) and wild oats (*Avena fatua* L.) are hosts of *F. graminearum* Group 1 and contribute to increased disease incidence (Atanasoff, 1920; Jenkinson & Parry, 1994).

1.4.2 Chemical control

Most wheat cultivars are susceptible to *F. graminearum* and one method of managing this disease is through the application of fungicides during anthesis (Yin *et al.,* 2009). In South Africa there are currently no chemicals registered for the control of FHB on wheat and barley (CropLife™ South Africa, 2009). Several fungicides are registered in the United Kingdom (U.K.) and U.S.A, but not all of these compounds are completely effective or consistent in their control and the reason for this may be the timing of application (Mesterházy, 2003a; Steffenson, 2003). Timing and rate of application are crucial to prevent *Fusarium* infection in the field. The earliest time to spray is after all heads have emerged and also during flowering stage (Mesterházy, 2003a; McMullen *et al.*, 2008). There are some reports describing successful chemical control of FHB that include carbendazim, hexaconazole, hexaconazole and carbendazim, mancozeb and benomyl, prochloraz, propiconazole, tebuconazole and triadimenol (Parry *et al.*, 1995; Doohan & Nicholson, 1996; Mesterházy, 2003a). None of these

fungicides resulted in excellent disease control but tebuconazole and prochloraz were the two most effective fungicides in greenhouse and field trials (Parry *et al.*, 1995; Mesterházy, 2003a). Paul *et al.* (2008) stated that fungicides should be useful when weather or other cultural conditions are favourable for disease development. Currently tebuconazole fungicides are generally the most effective in reducing FHB and DON at moderate disease and toxin levels. Other triazole based fungicides also proved to be efficacious in reducing FHB and DON, although metconazole and prothioconazole (both alone and in combination with tebuconazole) were consistently more effective than tebuconazole (McMullen *et al*., 2008; Paul *et al*., 2008). Application studies have shown that spray coverage and disease control with these fungicides are enhanced when sprays are directed at an angle either both forward and backward toward the grain head or with single nozzles directed toward the grain head, all at a 30° angle (McMullen *et al*., 2008).

The fungicide Prosaro^{TM} has been registered against FHB and diseases such as stem rust, leaf rust, *Septoria* leaf and glume blotch and tan spot, in the U.S.A. (http://www.aces.edu/timelyinfo/PlantPathology/2009/ March/pp670.pdf 29/09/2009).

In wheat growing areas where average yields are low (e.g. in developing countries), fungicide treatment is not economically feasible and did not reliably reduce DON concentrations to commercially acceptable levels (Mesterházy, 1997a). Fungicide application late in the heading stage may sometimes lead to residues on the harvested grain. If the harvested seed contains residues, the malting and food industries will not accept it (Parry *et al*., 1995; Steffenson, 2003).

Seed treatmens such as benomyl, mancozeb, maneb and thiram, can prevent seed and seedling infection and reduce the spread of seed-borne inoculum. This will improve the germination of seed and increase seedling vigour and yield (Gilbert & Tekauz, 1995). If scabby grain is to be used as a seed source it should be cleaned thoroughly to remove the majority of scabby kernels. A germination test should be done to ensure that the germination percentage is up to standard (McMullen *et al*., 2008). Although seed-treatments may offer protection to seed, it will have little or no effect on head blight because of the large amount of inoculum that can infect plants in the growing season (Wiese, 1987, McMullen *et al*., 2008).

1.4.3 Biological control

In addition to chemical control, there is an increasing interest in the use of biological agents to manage FHB. These agents include the application of micro-organisms such as bacteria and yeasts. Several microbial antagonists of *F. graminearum* have been identified which may be combined with chemical fungicides to reduce the amount of infection and DON contamination (Da Luz *et al.*, 2003). Micro-organisms with potential to control *F. graminearum* on wheat and barley includes bacteria such as *Bacillus* spp., yeasts such as *Cryptococcus* spp. and filamentous fungi such as *Trichoderma* spp. (Kahn *et al.*, 1998; Luo & Bleakley, 1999; Gilbert & Tekauz, 2000; Jochum & Yuen, 2001).

Other strategies for biological control include the disruption of the fungal life cycle. Biological interventions must be aimed at disruption of spikelet infection and the movement of *Fusarium* within the rachis and reducing the survival of the fungus in cereal debris with subsequent ascospore production (Da Luz *et al.*, 2003).

Currently methods to control FHB are only partially effective. Biological control may play an important role in integrated management of FHB because several microbial antagonists of *F. graminearum* have been identified with significant potential to reduce FHB. Biological control agents must have the ability to be produced on a large scale, have a long shelf-life, be efficient, grow and survive in the environment as well as be compatible with agricultural practices and implements to be successful in the market (Da Luz *et al.*, 2003; Gilbert & Fernando, 2004).

1.4.4 Resistance

According to Schroeder and Christensen (1963), Arthur associated FHB infection with a specific stage of wheat head development. The difference in flowering time might influence the amount of *Fusarium* infections and early maturing wheat cultivars are less susceptible to FHB than cultivars that matured later in the wheat season. Resistance to FHB has been categorized according to specific types which are summarized in Table 1.2.

Table 1.2: Types of resistance to FHB are categorized as indicated (Mesterházy, 2003b).

Type	Description
I	Resistance to initial infection (incidence)
II	Resistance to the spread of the fungus within the plant (severity)
III	Resistance to kernel infection. The rates of seed infection can differ at a given level of resistance as measured by disease severity
IV	Tolerance to FHB where tolerant wheat maintain yield despite of the presence of the disease
V	Resistance against toxin accumulation

Beyond Type I and II resistance there is little agreement on the numbers used to designate the types of resistance (Shroeder & Christensen, 1963; Bushnell *et al.*, 2003; Steffenson, 2003; Mesterházy, 2003b).

The best known sources of resistance are for Type I. An indicator for Type I resistance is the incidence of infection in trials using airborne inoculum and favourable environmental conditions. The indicator for Type II resistance is the single floret or single spikelet inoculation method which distinguishes it from Type I resistance (Stack, 1997; Miedaner *et al.*, 2003). Type II resistance is the most commonly reported type of resistance represented in the wheat cultivars "Sumai #3" and “Nuy Bay” (Bai & Shaner, 1994; Fedak *et al.*, 2007). With conventional breeding it takes up to 15 years for a resistant cultivar to be developed with all the desirable agronomic and quality traits because of the complexity of the task. It is thus a time consuming and expensive process (Steffenson, 2003). Another resistant cultivar is Frontana, which originated in Brazil and has the pedigree "Fronteira"/"Mentana". It is also resistant against leaf and stripe rust (Singh *et al*, 1995; Jiang *et al.*, 2006).

To accelerate breeding efforts, double haploids are being used to more rapidly achieve homozygosity in selected populations (Rudd *et al.*, 2001).

Evaluation for FHB resistance in phenotypes is resource and time intensive. Results are often confounded by environmental factors and needs to be repeated over environments. Molecular markers may provide new sources for identifying FHB resistance genes (QTL) in breeding populations. Although many QTL have been identified for FHB resistance, the one on 3BS has a major effect on resistance (Hongxiang *et al*., 2008).

1.5 SCREENING TECHNIQUES

There are strong debates concerning the most effective inoculation method for FHB assessment. Inoculation techniques differ between programmes internationally (Rudd *et al*., 2001). Some of the more general inoculation techniques include single floret inoculation, spray inoculation of the wheat head with a liquid spore suspension, the distribution of infected grain (grain spawn) or other plant material and solid media. Single floret inoculation is used to control the method of inoculation so that the initial inoculation point is limited to that single floret within one wheat head. This can be done using a pipette or syringe to inject a water suspension of spores into a single central floret at anthesis of the spike (Schroeder & Christensen, 1963; Engle *et al.*, 2003b). Inoculation is usually done with *Fusarium* macroconidia at concentrations of 10 000-100 000 macroconidia per ml (Gilbert & Woods, 2006; Somers *et al*., 2006).

A variation of this method is to place a small cotton wool ball, soaked in a suspension of *F. graminearum* macroconidia, into a central floret of a spike (Singh *et al*., 1995), (see Fig. 1.6) and is comparable to inoculation by injection with a syringe. The cotton wool method, however, does not injure the wheat head as is the case with the injection. According to Bekele (1984) this is the most precise method to use in a controlled FHB study in the greenhouse and the field.

Figure 1.6: After being soaked in a spore suspension of *F. graminearum,* a cotton wool wad was inserted into the middle of the wheat head. Note the discolouration of the florets.

Inoculated heads can be covered with bags for a 24 h period (Teich & Michelutti, 1993), or a misting system may be used for as long as 72 h (Mesterházy, 1997b; Rudd *et al.*, 2001). Inoculated spikes are visually rated for disease incidence and severity (Argyris *et al.*, 2005).

Spray inoculation involves the application of inoculum with an atomiser or artist's airbrush in the greenhouse, while a backpack sprayer, mist blower or a motor-driven sprayer is used to apply the spore suspension for field inoculations. An aqueous solution of macroconidia or ascospores is uniformly sprayed onto the heads, after which a moist period is provided to facilitate infection. Overhead irrigation can be used in the field and a mist chamber is used for greenhouse inoculations. This inoculation technique is designated to assess resistance to initial infection (Type I) (Schroeder & Christensen, 1963; Rudd *et al.*, 2001; Engle *et al.*, 2003b; Steffenson, 2003; Fuentes *et al.*, 2005; Gilbert & Woods, 2006; Klahr *et al.*, 2007). Spray inoculations can also be used to evaluate large numbers of material in the field or greenhouse. In field trials, plants are sprayed at 50% anthesis. Inoculation is usually repeated one week later to include later

developing spikes. Grain is harvested at maturity and evaluated for diseased kernel frequency and DON concentration (Rudd *et al.*, 2001).

Grain spawn inoculum is also used for the evaluation of large numbers of plants in the field (Rudd *et al.*, 2001). Typical grain used for field inoculation includes colonised maize, oats (*Avena sativa* L.), barley or wheat seed (Dill-Macky, 2003). The colonized grain is spread in plots at stem elongation (Zadoks growth stage 30 to 35) along the base of the plants and can be applied as a single or a split application, three weeks apart (Tottman, 1987; Dill-Macky, 2003; Markell & Francl, 2003).

A misting system can be used to increase the relative humidity, with misting periods varying between 12 and 72 h, although it is customary to keep inoculated heads in a saturated atmosphere for 72 h after inoculation. These conditions probably occur rarely in nature where wheat is grown. A delayed moist period will result in only 15 to 30% of heads developing the disease (Fuentes *et al*., 2005). Bleached and/or discoloured heads are visually rated 21 and 28 days post inoculation to determine the disease incidence and severity (Argyris *et al.*, 2005).

Overhead irrigation is used during the evening, night or early morning to enhance the formation of perithecia. Ascospores develop in the perithecia and are forcibly discharged from the asci and carried upward onto spikes by wind currents (Rudd *et al.*, 2001; Steffenson, 2003).

Other assessment techniques such as yield components (1000 grain weight and total yield), are the most effective way of identifying resistant cultivars under low inoculum pressure (Ireta & Gilchrist, 1994). According to Parry *et al.* (1995) the percentage of infected seed is the best way to identify resistant cultivars under low infection rates.

1.6 RATING SYSTEMS

Several different rating systems and scales have been used to assess *Fusarium* infection, leading to confusion. Stack & McMullen (1998) used a modified Horsfall-Barrett scale with ten categories of infection. This is frequently used for visual assessment of the percentage of diseased spikelets per head. To determine the percentage infection in the field, a representative area well

away from field edges and irregularities, must be chosen. Twenty to 30 heads per plot should be evaluated according to the modified Horsfall-Barrett scale, and repeated at least four to six times to reduce the amount of variability. The average scores (including all the zeros) give you an average plot severity. The number of infected heads divided by the total number scored gives the incidence of disease.

Another assessment scale can be used to estimate the proportion of diseased spikelets on heads in the greenhouse or field (Fig. 1.7). This scale can improve consistency of data obtained by multiple individuals conducting disease assessments on both wheat and barley (Engle *et al.*, 2003a; Klahr *et al.*, 2007).

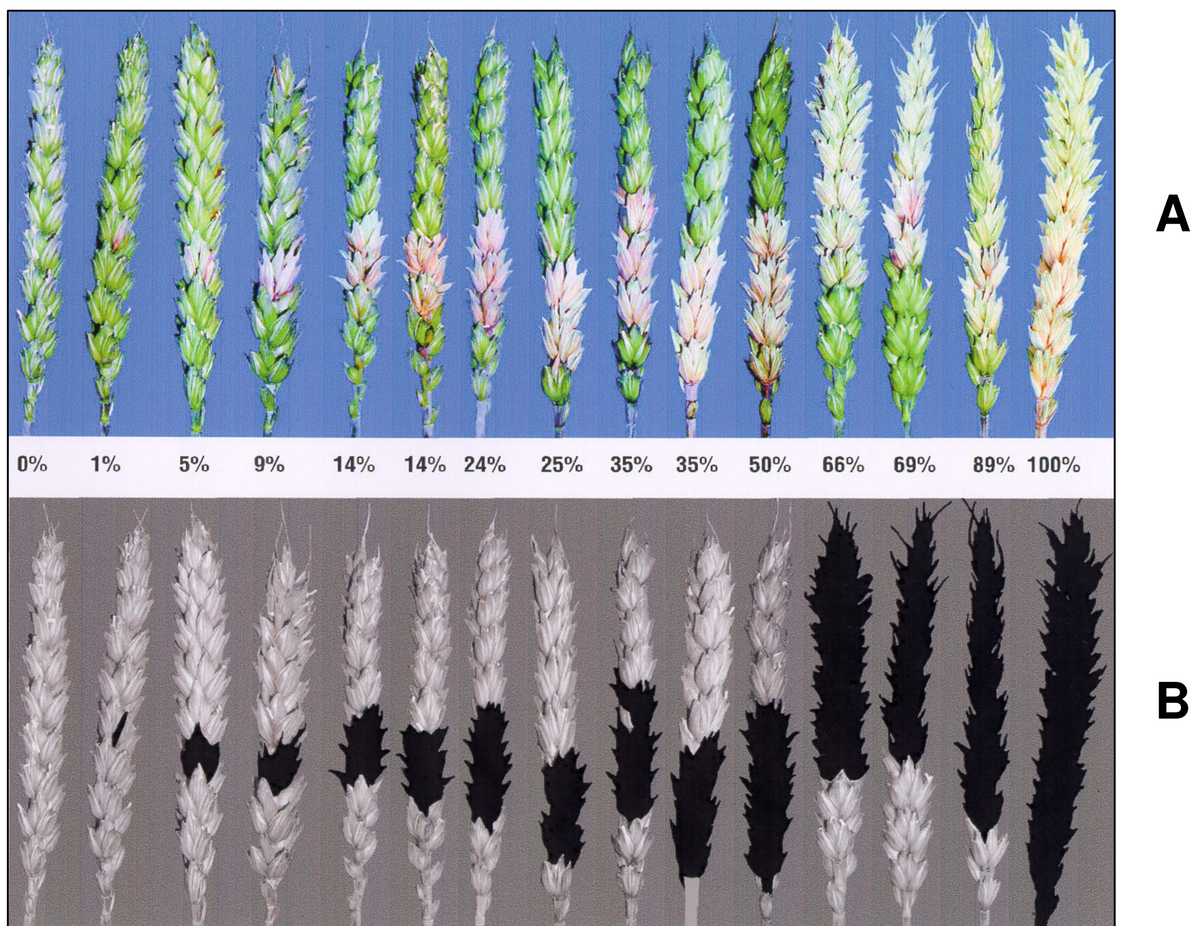

Figure 1.7: FHB severity scale, colour (A) and black and white (B) images of infected wheat heads, showing the percentages of diseased spikelets (Engle *et al.*, 2003a).

Mesterházy (1987) made evaluations in the field on a scale of 0 - 4, estimating the numbers of bleached heads in a group of 15 - 20 heads. These heads were evaluated on the 10th, 14th, 18th, 23rd and for later genotypes, also on

the 28th day after inoculation. Ten heads were selected from a group with average infection severity and heads were threshed after ripening, the seed were weighed and counted.

A method to determine the proportion of visually scabby kernels in harvested grain was developed to estimate the percentage of visually scabby kernels by matching a 100 gram grain sample with standards. The standards consist of 0, 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45 and 50% were generated by mixing healthy and scabby kernels (Dill-Macky, 2003).

Disease incidence has been used to quantify disease when natural infection occurs or where heads were inoculated. Disease severity is measured by counting the number of infected spikelets per head after inoculation. However, disease incidence multiplied by disease severity will give a disease index, which is useful to determine the host reaction to FHB (Dill-Macky, 2003).

1.7 CONCLUSIONS

FHB is one of the most destructive and common diseases of wheat and other cereals internationally. The first report of FHB was in England in 1884, although this disease is more important in warm and humid areas. It is caused by several species of the genus *Fusarium*, with three dominating species; *F. graminearum (Gibberella zeae), F. culmorum* and *F. avenaceum (G. avenacea).* The infection process begins with brown watery soaked lesions followed by bleaching of parts of the head. Environmental conditions must be favourable for the disease to develop. These conditions include high humidity and rainfall during flowering and grain fill period. In South Africa, yield losses of up to 26% may occur in heavy infected fields and will include sterile heads and non-viable or shriveled seed (Scott *et al.*, 1988). Although this percentage can be lower, the accumulation of mycotoxins such as DON, NIV and ZEA, has a huge impact on food industries.

The primary factors contributing to epidemics are changes in cultivation practices, together with the extensive cultivation of susceptible cultivars and favourable environmental conditions. Sporadic occurrence of FHB remains another issue and for this reason a breeding programme based only on natural infection, is not always possible.

FHB is difficult to control, so it is imperative to prevent the disease from becoming established in a field. Minimizing FHB can only be achieved through an integrated approach including cultivation practices, fungicide application and the use of resistant cultivars. Small grain disease forecast models are valuable tools to alert farmers of possible disease outbreaks. Breeding for FHB resistance can be done, but it is a difficult and time-consuming process. To date, the most widely used and recognized source of resistance is from the Chinese cultivar Sumai #3, and identification and verification of other sources of resistance to FHB is crucial to enable breeders to combine sources of resistance for more effective resistance in breeding programmes. Field evaluations are usually conducted only once a year, so breeding programmes have to rely on greenhouse evaluations to achieve rapid progress towards the development of resistant germplasm. It has to be considered though, that assessing resistance in the field is more reliable than testing in the greenhouse, because the environmental effects on resistance can not always be reproduced in the greenhouse.

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CHAPTER 2

VIRULENCE AND SPORULATION ASSESSMENT OF SOUTH AFRICAN ISOLATES OF *FUSARIUM GRAMINEARUM*

2.1 INTRODUCTION

Fusarium head blight (FHB), also known as scab, is one of the most destructive diseases of wheat (*Triticum aestivum* L.) and other small grains worldwide (Wiese, 1987; Parry *et al*., 1995; McMullen *et al*., 1997). *Fusarium graminearum* (*Gibberella zeae*) is the predominant causal organism on wheat and barley (*Hordeum vulgare* L.) (Parry *et al*., 1995; Bai & Shaner, 2004). In South Africa the disease is especially prevalent where wheat is grown under irrigation (Scott *et al*., 1988; Kriel & Pretorius, 2008). The disease is more severe when wheat is sown in the residue of a previous host crop such as maize (*Zea mays* L.), particularly under warm and humid conditions (Wiese, 1987; Bai & Shaner, 1994; Markell & Francl, 2003).

The disease is recognized by the appearance of one or more bleached wheat heads after flowering, whereas healthy plants remain green (Pomeranz, 1990). Pink to peach coloured mycelia and conidia form on infected kernels (Steffenson, 2003). Yield losses that include reduced kernel number, kernel weight and grain quality may cause losses of up to 100% (Schroeder & Christensen, 1963; Snijders, 1990; Bai & Shaner, 1994; Parry *et al*., 1995; McMullen *et al*., 1997; Nicholson *et al*., 2007). The diseased grain may also contain mycotoxins such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA), that may be hazardous to human and animal health (Sutton, 1982; Snijders, 1990; McMullen *et al*., 1997; Steffenson, 2003; Stein *et al*., 2009).

Genetic resistance has the potential to provide cost effective control of FHB, but dependable methods to determine resistance levels are needed. The best-known cultivar that has resistance to FHB is Sumai #3 (Chinese origin), an improved cultivar with good combining abilities for yield and FHB resistance (McKendry, 2000; Bai & Shaner, 2003; Mesterházy, 2003). This cultivar has been successfully used as a resistant parent in wheat breeding programmes worldwide (Cook, 1981; Bai & Shaner, 2003). Two Brazilian cultivars namely Frontana and Encruzilhada have also been reported to possess FHB resistance and are therefore utilized in breeding programmes (Bai & Shaner, 2004).

Resistance in wheat to *F. graminearum* has been characterized using various tests that might differentiate among the different types of resistance (Schroeder & Christensen, 1963; Bai & Shaner, 1994). Resistance to FHB can be categorized according to the specific expression of the resistance (see Chapter 1).

There are a number of different inoculation techniques available and the technique of choice differs among programmes internationally (Rudd *et al.*, 2001). Some of the more common artificial inoculation techniques include single floret inoculation, spray inoculation of the wheat head with a liquid spore suspension, the distribution of infected grain (grain spawn) or other substrates, as well as different solid media used to culture the fungus (Buerstmayr *et al.*, 2000).

Various attempts have been made to improve media for sporulation of *Fusarium spp.* and the effect of different compounds on growth and enumeration has been studied to produce macroconidia for field or greenhouse evaluations. Some of these media include carboxymethyl cellulose (CMC) liquid medium (Cappellini & Peterson, 1965; Gilbert & Woods, 2006), mung bean medium (Evans *et al.*, 2000; Dill-Macky, 2003), carnation leaf agar (CLA) (Fisher *et al.*, 1982; Dill-Macky, 2003) and carrot agar (CA), (Dhingra & Sinclair, 1985).

Different screening techniques have been developed to test Type I and Type II resistance in the greenhouse and field. Type I resistance is evaluated by spraying heads with a macro-conidial suspension, using a hand atomizer, until runoff. Type II resistance is evaluated by various inoculation methods such as injecting a macro-conidial suspension into a single central floret using a hypodermic syringe or a micropipette (Schroeder & Christensen, 1963; Mesterházy, 2003). Another method is to place a small cotton wool ball, soaked in a macroconidia suspension, in a central floret of a wheat head (Gilchrist *et al.*, 1996; Van Ginkel *et al.*, 1996; Ntushelo & Boshoff, 2002; Steffenson, 2003). The advantage of the injection method is that it is precise and reliable since the inoculum can be applied quantitatively. The disadvantages are that the needle may injure the wheat tissue and the inoculum may drip, which will contaminate other parts of the head (Bekele, 1984). The advantage of the cotton wool method is that it gives more controlled inoculation of a wheat head so the initial inoculation point is restricted to a single floret within one head (Schroeder & Christensen, 1963; Engle *et al.*, 2003). Another advantage of the cotton wool

method is that no injury of the wheat head occurs during inoculation as in the case of injecting the spore suspension (Bekele, 1984).

Gilbert & Woods (2006) reported that each inoculated floret is marked with a non-toxic pen. The *Fusarium* macroconidia may be applied at concentrations ranging from 10 000 to 100 000 macroconidia per milliliter. The commonly recommended concentrations for inoculation is 50 000 per ml (Somers *et al.*, 2006). Assessing resistance in the field is possible but environmental conditions generally cannot be manipulated. Field evaluations can only be conducted once a year, therefore breeding programmes rely on the use of greenhouse techniques for screening breeding and resistance donor materials.

This study was conducted to achieve the following objectives:

1. Testing the virulence of *F. graminearum* isolates in the greenhouse against a susceptible cultivar.
2. Select five of the most virulent isolates from this trial and do a laboratory assay to determine the medium that will produce the most macroconidia over time.

2.2 MATERIAL AND METHODS

2.2.1 Experiment 1: Virulence testing

2.2.1.1 Plant material and isolates

The irrigation spring wheat cultivar SST825 was used in a greenhouse study to determine the most virulent *F. graminearum* isolate out of the 20 accessions obtained from the University of the Free State collection. Originally, the isolates were all obtained from wheat in the Prieska region and included cultures A144, A145, A146, A149, A151, A156, A159, A161, A166, A167, A169, A176, A183, A187, A191, A194, A204, A221, A223 and A269. Each replication included individual heads inoculated with cotton wool soaked in a spore suspension. Five FHB assessments were carried out with the final evaluation 21 days after inoculation.

2.2.1.2 Trial preparation in greenhouse

Seeds of SST825 were planted in two-liter capacity containers in steamed sterilized soil/Culterra potting soil mixture (50/50) in a greenhouse. Eight seeds per pot were initially planted and after emergence the plants were thinned to five plants per pot. Greenhouse temperatures were set at 18℃±2℃ (night) and 22℃±2℃ (day). The trial was automatically irrigated three times a day. Fertilizer was applied from Zadoks growth stage (GS) 13 up to 55 (Tottman, 1987). Fertilizer included LAN (limestone ammonium nitrate, N-28%) and Supafeed® (Plaaskem Pty. Ltd.) a water-soluble fertilizer for foliar feed and fertigation. Supafeed® includes N (15%), P (4.5%), K (26.3%) and other spore elements such as Mg, Zn, B, Fe, Mn and Cu. Both LAN and Supafeed® were given every fortnight. No fungicides were applied in the greenhouse, but insecticides were sprayed twice to control aphids during GS 19 (9 or more leaves unfolded) and GS 32 (2nd node detectible).

2.2.1.3 Production of inoculum

The 20 *F. graminearum* isolates were cultured on mung bean medium. Each isolate was cultured on 20 Petri dishes for four weeks at 22℃ in an incubator. Mycelium from each Petri dish was harvested using the following method: 30 ml of distilled water was added to a Petri dish and the mycelia were harvested with a sterile L-shape glass rod and poured into a flask. The mixture was sieved through two layers of cheesecloth and the spore concentration of each isolate was determined before inoculation (Table 2.1). After harvesting, the spore suspension was stored at 4℃ for future use.

Table 2.1 ***Fusarium graminearum*** **isolates and their spore concentrations (count/ml x10^4) used for inoculations in the greenhouse trial.**

ISOLATE	SPORE CONCENTRATION (per ml)
A144	01.25
A145	09.00
A146	04.75
A149	02.50
A151	10.75
A156	01.00
A159	03.50
A161	05.00
A166	02.75
A167	01.00
A169	08.25
A176	05.00
A183	05.00
A187	12.25
A191	09.75
A194	00.50
A204	07.50
A221	01.00
A223	00.75
A269	02.00

2.2.1.4 Inoculation time and methods

Inoculations were done just before or during anthesis at GS 60 and 61. At this stage the wheat spike is the most susceptible to *Fusarium* (Grausgruber *et al.,* 1995*).* The cotton wool method consists of 3 mm diameter wads of cotton wool, soaked in the suspension of inoculum and inserted into a single floret in the middle of a wheat head. Each inoculated head was tagged with the appropriate treatment details (Fig. 2.1).

Four replications with 20 heads per replication for each isolate were included in the trial along with a clean control, inoculated with wads of cotton wool soaked in distilled water.

Figure 2.1: Plants were tagged with masking tape showing all relevant treatment details.

After inoculation, plants were placed in a dew chamber for 18 h at 22℃±2℃ with 98.5% relative humidity (RH). After 18 h the plants were removed and returned to the greenhouse.

2.2.1.5 Evaluation of the disease

To determine the progress of infection, disease symptoms were evaluated 7, 10, 14, 17 and 21 days post-inoculation. The severity of the infections was recorded by counting the number of bleached florets in relation to the total number of florets and expressed as a percentage.

2.2.1.6 Data handling and statistical analysis

The experiment was set up as a complete randomized block using twenty isolates and one uninfected control that were each replicated four times. Each replication consisted of twenty individual heads. Five evaluations were carried out with the final evaluation 21 days after inoculation.

The AUDPC was calculated using the following equation: (Shaner & Finney, 1977; Campbell & Madden, 1990; Buerstmayr *et al.*, 2000; Jeger & Viljanen-Rollinson, 2001):

$$AUDPC = \sum_{i}^{n-1} \{[(y_i - y_{i-1})/2] \ (t_i - t_{i-1})\}$$

(where: n = total number of observations, y_i = visually infected spikelets on the ith day and t_i is the day of the ith observation).

These results were used for further statistical analysis. ANOVA was performed using the statistical program GenStat®, Release 10 (Payne, 2007) to determine:

- the statistical differences between the isolates,
- the least significant differences at a 5% confidence level, and
- the coefficient of variance (CV) of the trial.

2.2.2 Experiment 2: Inoculum production

2.2.2.1 Isolates

The five isolates that caused most FHB in the greenhouse were used for this study. These isolates, originally isolated from wheat in the Prieska region in South Africa, were all preserved as mycelial plugs in 30% glycerol and stored at -80℃ for further study.

2.2.2.2 Microbial culture media

The following culture media were used and a detailed preparation method is described in Annexure A:

- Carrot agar (CA; Dhingra & Sinclair, 1985),
- Potato dextrose agar (PDA; Dhingra & Sinclair, 1985),
- Malt extract agar (MEA) containing 5 g sterile dry maize material (Gilchrist-Saavedra *et al.*, 1997), and
- Mung bean agar (MBA) (R. Dill-Macky, e-mail communication).

2.2.2.3 Preparation of isolates

The five isolates were initially prepared on PDA and after eight days of growth, aseptically transferred to CA, MBA, MEA and PDA Petri dishes. A plug of 6 mm in diameter was extracted from the PDA Petri dish for the transferal. Twenty-five Petri dishes per isolate and per medium were used for this test and all Petri dishes were placed in an incubator at 22℃±1℃.

2.2.2.4 Colony growth of isolates

The colony growth of each isolate was measured on a linear line from day one to day seven and growth rate was calculated in millimeters per 24 h. After seven days the total growth of each isolate was determined (Fig. 2.2).

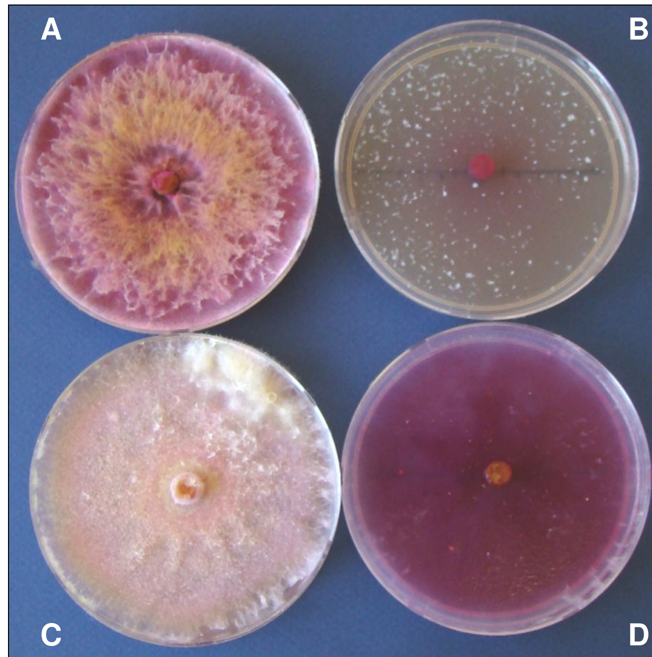

Figure 2.2: Fully grown Petri dishes of isolate A191, A: MEA; B: CA; C: PDA; D: MBA.

2.2.2.5 Spore counting

Spore counting was done by using a Neubauer haemocytometer. Three ml water was added to each of the five Petri dishes that were used per isolate per week. The mycelial growth on the agar was well mixed with the water using an L-shape glass rod. The suspension of five Petri dishes per isolate per medium was scraped off and sieved through two layers of cheese cloth. This spore suspension was used for the haemocytometer counts. Two drops of this spore suspension were placed on a haemocytometer, covered by a cover slip and the spores counted using a light microscope. For each isolate, five spore counts were done to determine the mean number of spores per ml.

The remaining Petri dishes were allowed to grow for another week before another five Petri dishes per isolate were harvested for the second count. Isolates were harvested in the same manner as described above and harvesting of spores continued every week up until week four.

2.2.2.6 Data analysis for the greenhouse and laboratory trials

Data for the growth and spore counts of the isolates were analysed using the statistical program Genstat® Release 10 (Payne, 2007) and an ANOVA was used to determine the LSD at 1% between media, isolates and their interaction.

2.3 RESULTS AND DISCUSSION

2.3.1 Experiment 1: Virulence testing

2.3.1.1 Ranking

The virulence of the tested isolates is shown in Fig. 2.3. It was noted that isolate A191 had the highest disease development overall and isolate A187 had the lowest disease development.

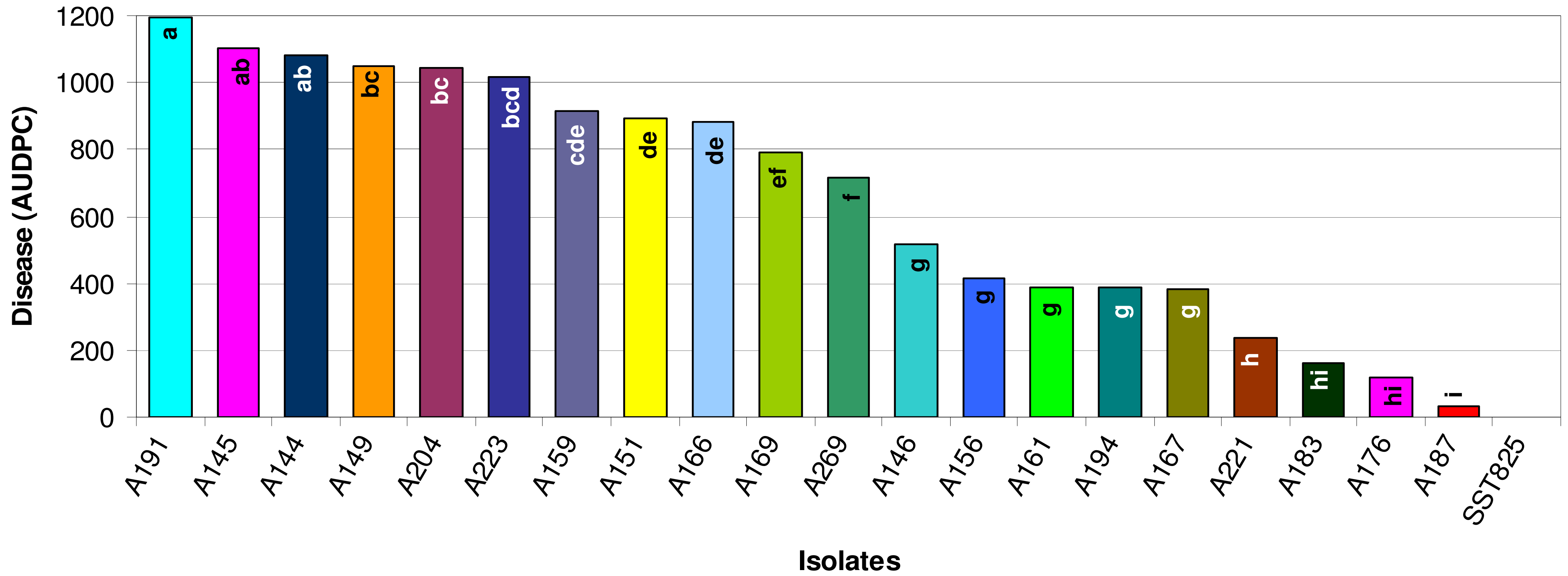

Figure 2.3: Virulence of different *F. graminearum* isolates on SST825, tested in the greenhouse.

The cotton wool method, as previously described, is currently the best method in assessing Type II resistance in the greenhouse (Van Ginkel *et al.*, 1996; Ntushelo & Boshoff, 2002; Steffenson, 2003) and is comparable to inoculation by injection. According to Bekele (1984) the cotton wool method is the most precise method to use in a controlled study. For disease development warm temperatures (above 15℃) and humid conditions are needed (Parry *et al.*, 1995).

Figure 2.3 shows significant differences in AUDPC between the isolates tested. The mean AUDPC of isolate A191 was 1192, the mean of isolate A145 was 1105 and the mean of isolate A144 was 1082. Isolate A191 differed significantly from isolate A149. It is evident that isolate A191 caused more FHB although it did not differ significantly from isolate A145 and isolate A144. Isolates A221, A183, A176 and A187 showed the lowest disease development over time and did not differ significantly from each other. The least significant difference of means (LSD) at 5% confidence level was 140 with a 14.8% CV.

Large differences in pathogenicity among isolates are well known and evidence of interactions between cultivars and pathogen isolates can also be seen in some greenhouse trials (Dill-Macky, 2003; Mesterházy *et al.*, 2005). Isolate A191, which had an original spore concentration of 9.75×10^4 per ml, caused most FHB as opposed to isolate A187 which, despite a spore concentration of 12.75×10^4 per ml, performed the worst of all the isolates in the virulence test. The coefficient of determination (R^2) between AUDPC and spore concentration was 0.0085. This confirmed that spore production is not a reliable variable in virulence expression (Shaner, 2003).

From the 20 isolates used in the greenhouse study, the five most virulent isolates, A144, A145, A149, A191 and A204, were chosen to determine the best solid medium to induce vigorous sporulation for inoculum production.

2.3.2 Experiment 2: Inoculum production

2.3.2.1 Growth of the different isolates

Fusarium needs favourable environmental conditions for good sporulation. These conditions include sufficient light, temperature and a beneficial growth medium (Toussoun & Nelson, 1976).

The average growth of the five isolates on the different agar media from day one up to day seven can be seen in Fig. 2.4. In Fig. 2.5 the isolate growth on the media over seven days can be seen and Fig. 2.6 shows the interaction between the isolates and agar media.

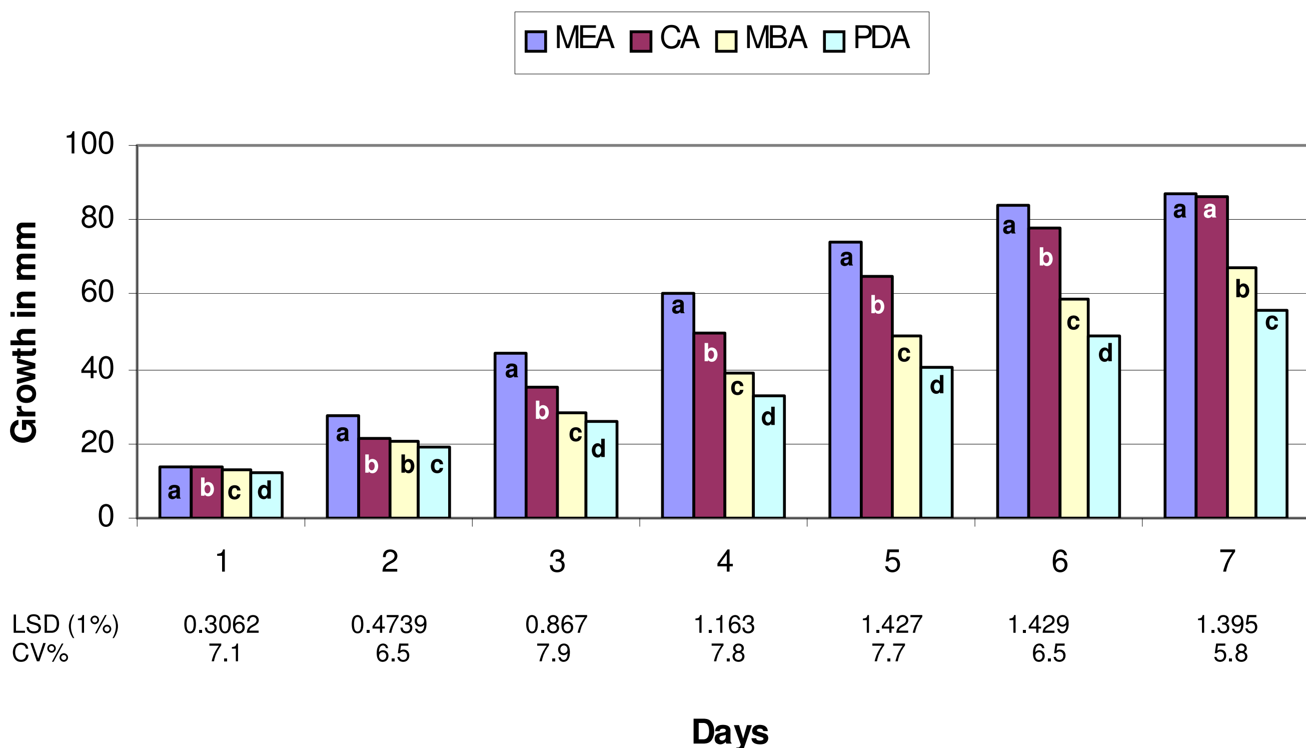

Figure 2.4: Growth of isolates on different agar over a seven day period.

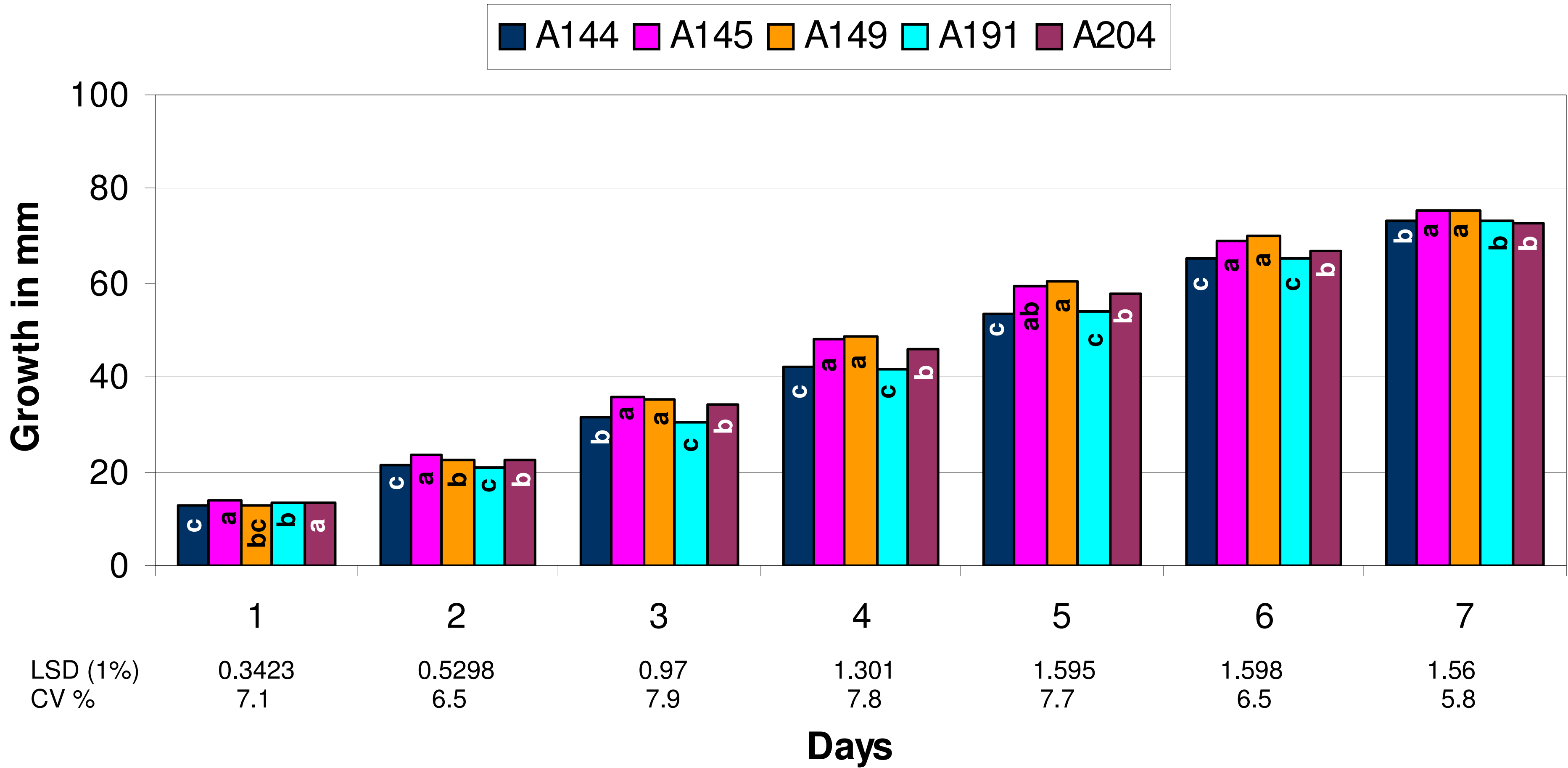

Figure 2.5: Growth of different isolates over a seven day period.

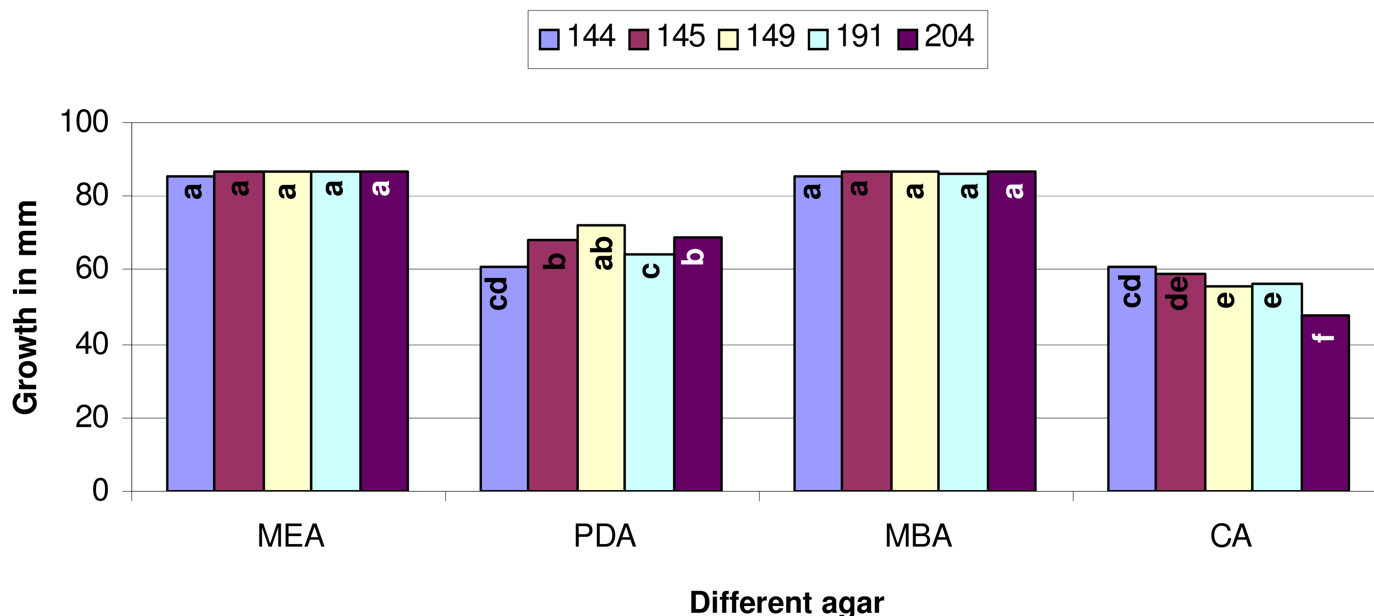

Figure 2.6: Growth of the different isolates on the different media on day seven.

As can be seen from Fig. 2.4, no significant differences between the media occurred between days 1 to day 6. The only significant difference on day 7 was between PDA and CA and no significant differences were observed between MEA and MBA.

Figure 2.5 shows the isolate growth on the different media over a seven day period. No significant differences occurred between isolates A145 and A204, but isolate A191 differed significantly from A144. On day 4 isolate A149 and A145 as well as isolates A144 and A191 did not differ significantly from each other, but differed significantly from isolate A204. On day 7 the trend was generally the same, whereas isolates A149 and A145 differed significantly from isolates A191, A144 and A204. This showed that the growth of isolates A149 and A145 was the best at day 7.

In Fig. 2.6 the isolate growth is shown on all the different media at day 7. It is clear that MEA and MBA were excellent media to use. PDA and CA differed significantly from MEA and MBA. The LSD (1%) for day 7 was 3.119 and the CV was 5.8%.

From the above tables it is clear that MEA performed the best for growth of all the isolates. The Petri dishes were completely overgrown between six to seven days. CA as well as PDA will not be recommended to

use for a growth medium for these specific isolates, whereas MEA and MBA can be used to maintain *Fusarium* cultures.

2.3.2.2 Sporulation of different isolates

The spore counts on the different media were done over time with an improved Neubauer haemocytometer and the data for four weeks are shown in Table 2.2.

Table 2.2: The mean spore count per ml on different agar media over a four week period.

Note: MEA = Malt extract agar; CA = Carrot agar; MBA = Mung bean agar;

NEUBAUER	MEA	CA	MBA	PDA
Week 1	3.10×10^{4} h	2.02×10^{4} ij	6.52×10^{4} f	0.38×10^{4} k
Week 2	1.44×10^{4} ij	4.28×10^{4} g	32.66×10^{4} d	0.40×10^{4} k
Week 3	1.12×10^{4} j	25.00×10^{4} e	125.36×10^{4} b	0.12×10^{4} k
Week 4	3.52×10^{4} hi	62.94×10^{4} c	255.76×10^{4} a	0.28×10^{4} k

PDA = Potato dextrose agar

Significant differences occurred between the spore counts done of the various media over the four week period (Table 2.2). MBA showed a higher number of spores produced than CA, MEA and PDA. The high spore count on MBA was to be expected as it is one of the best mediums to use for sporulation (R. Dill-Macky, e-mail communication) and also inexpensive to prepare (Gilchrist-Saavedra *et al.*, 1997).

Carrot agar is the second best medium to use although it has been described as a poor medium to induce sporulation in many fungi (Dhingra & Sinclair, 1985; Chen *et al.*, 2009). Another use of CA is in fertility studies of *Fusarium* species with a *Gibberella* teleomorph (Chen *et al.*, 2009).

In week 4 the lowest spore numbers occurred on MEA and PDA with counts of 3.52×10^4 and 0.28×10^4, respectively. This was expected since MEA and PDA are rich media, widely used for isolating, multiplying and storing of fungi, but not sporulation (Jones & Clifford, 1983). PDA may also be used to assess pigmentation and gross colony morphology of mainly *Fusarium* spp. (Leslie & Summerell, 2006).

Malt extract (Biolab) and agar agar (Biolab) with the addition of 5 g/ℓ autoclaved chopped maize straw was used to induce spore formation (Gilchrist-Saavedra *et al.*, 1997; Leslie & Summerell, 2006). In this test the ME agar amended with maize straw did not improve spore production.

Haemocytometer counts are a general method to use for spore counting, since it is easy and cheap to use. The LSD (1%) was 41.68 and the CV was 16.9%.

In conclusion, it is clear that MBA is the best medium for spore production after four weeks and isolate A191 is the most virulent, differing significantly from the other isolates. A191 will be utilized in further studies to determine the best inoculation technique for Type I and Type II resistance in the greenhouse and field.

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CHAPTER 3

DEVELOPMENT OF AN EFFECTIVE INOCULATION TECHNIQUE TO TEST TYPE I AND TYPE II RESISTANCE AGAINST FHB IN THE GREENHOUSE AND FIELD

3.1 INTRODUCTION

Fusarium head blight (FHB), caused by *Fusarium graminearum* (teleomorph *Gibberella zeae* (Schw.) Petch) and other *Fusarium* spp., has become a major problem in more humid wheat (*Triticum aestivum* L.) production areas (Miedaner *et al*., 2003; Steffenson, 2003; Bai & Shaner, 2004; Trail, 2009). It was first recognized as a fungal disease in 1884 by W.G. Smith (Trail, 2009) and is a major threat to wheat and barley (*Hordeum vulgare* L.) industries worldwide.

The primary sources of inoculum are ascospores and conidia produced on wheat, maize or barley residues (Sutton, 1982; Sturz & Johnston, 1985; Windels *et al*., 1988; Markell & Francl, 2003). Initial infection of wheat heads occurs primarily at anthesis and is influenced by temperature and relative humidity (Andersen, 1948; Snijders, 1990). Seed infected by *Fusarium* may serve as a source of inoculum in areas that are still disease free and will also result in seedling blight and a subsequent reduction in seedling density (Atanasoff, 1920; Cook 1981). Crop residues on the soil are very important for the survival of the pathogen, resulting in up to three times higher levels of FHB in wheat fields sown in maize residue (Ireta & Gilchrist, 1994).

Yield reduction due to the production of shriveled kernels and lower grain quality from mycotoxin contamination present the biggest threat of FHB (Snijders, 1990; McMullen *et al*., 1997; Miedaner *et al*., 2003; Steffenson, 2003).

Various methods can be used to control *Fusarium,* such as appropriate land preparation as well as crop rotation, to assist in reducing primary inoculum (Andersen, 1948; Cook, 1981; Jones & Clifford, 1983; McMullen & Stack, 1999). Breeding of wheat for durable resistance against this disease is the most effective and economical means of reducing yield losses and mycotoxin contamination, but it is difficult due to confounding environmental conditions. The improvement of FHB resistance has become a major breeding

objective worldwide. There are five types of resistance (Mesterházy, 1995) as discussed in Chapter 1.

Different inoculation methods are used to infect field plots to be screened for FHB Type I resistance. Spray inoculation consist of applying an inoculum suspension with a backpack sprayer or mist blower. This procedure should be done at least twice, starting at the beginning of anthesis and ensuring that the suspension uniformly coats the wheat heads. A moist period must be provided to promote infection. This can be achieved by overhead irrigation (Buerstmayr *et al.*, 2000; Rudd *et al.*, 2001; Dill-Macky, 2003).

Grain spawn can also be used for field inoculations (Rudd *et al.*, 2001). Typical grain used for inoculum production includes maize, oats, barley or wheat (Dill-Macky, 2003). *Fusarium*-colonized grain are spread in plots along the base of the plants, at a rate of 10 g/m^2 (or 10 kg/ha), at stem elongation or Zadoks GS 30 to 35 (Tottman, 1987; Dill-Macky, 2003; Markell & Francl, 2003). Grain spawn can be applied either in a single or split application to ensure infection, with the second application applied up to three weeks after the first application (Dill-Macky, 2003). Overhead irrigation should be applied during the night or early morning to ensure the formation of perithecia in the grain inoculum within about ten days. Ascospores are forcibly discharged from the perithecia and carried upwards onto spikes by wind currents (Rudd *et al.*, 2001; Steffenson, 2003). Colonized grain inoculum can be used alone or in combination with spray applications (Rudd *et al.*, 2001; Dill-Macky, 2003). Type II resistance is evaluated by various other inoculation methods as described in Chapter 2.

Disease incidence on naturally infected or spray-inoculated heads, are evaluated by determining the percentage of heads exhibiting disease symptoms. This method is more suited to field than greenhouse evaluations (Dill-Macky, 2003). Disease severity refers to the proportion of spikelets on the head that is symptomatic. Incidence is then multiplied by the severity to give an estimation of the overall head blight intensity (FHB index) in a field (Shaner, 2003).

Environmental conditions have a huge influence on disease development and if prolonged humid weather persists after initial infection, severe FHB will occur. Favourable temperatures for the production of ascospores and macroconidia vary between 16℃ to 36℃. Temperatures for

the formation of perithecia vary between 16°C and 31°C, but the optimal reported temperature for perithecium formation is 29°C. Ultraviolet light shorter than 390 nm is needed for perithecial initiation and they will mature between nine to ten days under favourable conditions (Sutton, 1982). Secondary infection is possible after early disease outbreaks, although wheat heads become much more resistant after anthesis (Wiese, 1987; Shaner, 2003; Murray *et al.*, 2009). Head blight symptoms will develop after infection at temperatures ranging between 25°C to 32°C with a lower limit at 15°C (Sutton, 1982). Temperature is not the only primary weather condition that determines the severity of the disease, as precipitation appears to be critical in disease development. High relative humidity (RH) of between 12 – 15 h, accompanied by frequent rainfall, will be sufficient for a disease epidemic (Shaner, 2003).

The objectives for this study were to:

- Determine the best inoculation method of screening for FHB resistance (Experiment 1) and to screen a set of germplasm with unknown response to FHB in the greenhouse (Experiment 2).
- Perform trials over a two year period to determine FHB reactions under field conditions (Experiment 3).

3.2 MATERIALS AND METHODS

3.2.1 Plant material and preparation

3.2.1.1 Greenhouse trials:

Experiment 1:

A range of susceptible to resistant lines/cultivars were used for this trial to identify a suitable screening technique for Type I and Type II resistance against FHB in the greenhouse. Cultivars included SST822, SST825, Krokodil, Frontana and SUM3/3/CS/LE.RA//CS/4/YANGMAI158 (no. 18 of the 10th Scab Resistant Screening Nursery [SRSN], Annexure B).

The untreated seeds were planted in the greenhouse in two liter capacity containers in steamed sterilized soil/Culterra potting soil mixture (50/50). Eight seeds were initially planted per pot and after emergence the

number of plants was thinned to five. Greenhouse temperatures ranged between 18℃±2℃ and 22℃±2℃ night/day. The trial was automatically irrigated three times a day and fertilizer was applied weekly from Zadoks GS 13 up to 59. Fertilizer included LAN (limestone ammonium nitrate, N-28%) and Supafeed® (Plaaskem Pty. LTD.), a water-soluble, foliar fertilizer. LAN and Supafeed® were applied alternatively every fortnight. No fungicides were applied in the greenhouse, but chlorpyrifos was sprayed at GS 17 (7 leaves unfolded) to control aphids.

Experiment 2:

A germplasm set from CIMMYT, Mexico, was used to determine Type II resistance against FHB in the greenhouse. SST825, Sumai #3 and 13 lines from the 9th SRSN were used in this experiment (Annexure C). SST825 acted as susceptible and Sumai #3 as resistant controls, respectively. Plants were grown as described for the first experiment. No fungicides were applied in the greenhouse, but chlorpyriphos was sprayed at GS19 (9 or more leaves unfolded) and 32 (2nd node detectable) to control aphids.

3.2.1.2 Field trials:

Experiment 3:

Seventy-five lines from the 9th SRSN together with SST825, Sumai #3, SST806 and Marico were used in both field trials of 2007 and 2008. The 9th SRSN had already been tested under field conditions in Mexico and from this nursery selections were made. SST825 is a South African cultivar that is susceptible to FHB. Sumai #3 was used as a resistant (R) control, Marico as a moderately resistant (MR) control and SST806 as a susceptible (S) control (McKendry, 2000; Kriel & Pretorius, 2008).

A honeycomb design was used in both years (Lungu *et al.*, 1987; Fasoulas, 1988). A total of 15 seeds per entry were planted in hill plots under floppy irrigation at the Small Grain Institute, Bethlehem, on 16 July 2007 and on 18 July 2008, respectively. Entries were spaced 50 cm apart, except for the two controls that were planted next to each other as Fig. 3.1 indicates.

Fertilizer [6:2:1 (31) Zn; with N 207 g/kg; P 69 g/kg; K 34 g/kg)] was added at planting. The soil was fallow during the previous season.

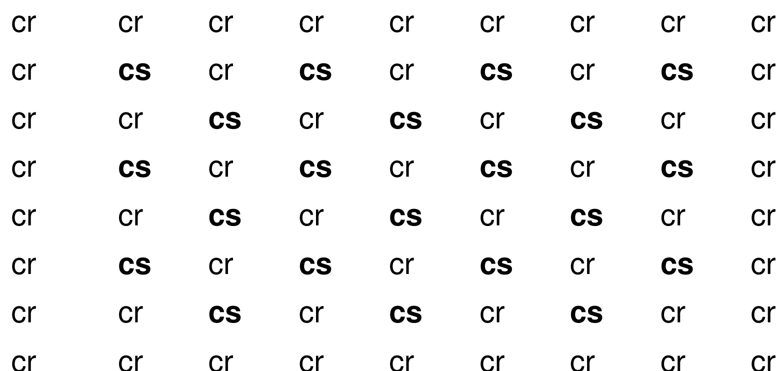

Figure 3.1: Layout of the honeycomb design used in the field, where CS indicates the entry and CR the two controls: SST806 (S) and Marico (MR).

Entries 25, 48, 49, 69 and 75 did not germinate in the 2007 planting. Entry 1 was a winter type and did not produce any heads. Entries that did not germinate in 2008 included nos. 24, 36, 47, 48 and 49. The trials were weeded by hand and no herbicides were sprayed.

Plants were irrigated on a weekly basis and fertilizer (LAN) was applied at GS 47 (730g on 45.36m^2 plot). No fungicides were applied but an insecticide (chlorpyriphos) was sprayed once at GS 32 in 2007 to control aphids. No insecticides were sprayed during 2008. In 2007, overhead irrigation was applied on a weekly basis until GS 55 where-after regular rain occurred until harvest. In 2008 irrigation was applied until the soft dough stage (GS 85) where-after rainfall was sufficient.

The rainfall, relative humidity, day and night temperatures, as well as evapotranspiration (ETO), were measured throughout the wheat season until harvest for both seasons.

3.2.1.3 Inoculum production, intervals and methods

Greenhouse trials:

For both greenhouse trials *F. graminearum* isolate A191 was used. The isolate was cultured on 35 mung bean medium Petri dishes at 23°C for

four weeks before harvesting. Each Petri dish was harvested using the following method: 30 ml of distilled water was added to a Petri dish, the spores were suspended using a sterile L-shape glass rod, and poured into a flask. The mixture was sieved through two layers of cheesecloth and the spore concentration was determined at 1.75×10^5 for inoculation of the first experiment. For the second experiment the inoculum concentration was 1.4×10^5.

Both trials were inoculated during anthesis at GS 59 and 60. The wheat plant is the most susceptible to *Fusarium* at this stage (Grausgruber *et al*., 1995). Three inoculation methods were used in the first experiment including spray, cotton wool and straw methods. The spray method made use of an ABAC airbrush, model PAB1209, with a glass vial filled with the spore suspension and connected to an air compressor. This suspension was sprayed over the flowering wheat heads and left to dry before the plants were placed in a dew chamber. For the cotton wool method, 3 mm diameter wads of cotton wool were soaked in the spore suspension and inserted into a single floret in the middle of the head. After inoculation, plants were placed in a dew chamber for 18 h at 22℃±2℃ with 98.5% relative humidity (RH). Plants were removed from the dew chamber after 18 h and placed in the greenhouse in random order. For the straw method, a 2.5 cm piece of plastic straw, sealed on the one end, was used to remove a plug of fungal growth from a four-week old culture of A191 on mung bean agar. To inoculate the wheat head, a single floret in the middle of the wheat head was cut open and the straw containing the mung bean agar plug was inserted over the open floret (Fig. 3.2).

Figure 3.2: The straw technique: a straw section with a colonized agar plug was inserted over a floret.

Four replications per isolate with 20 heads per replication and a control were included in the trial. Control plants were inoculated with sterile distilled water in the case of spray and cotton wool inoculations. In the case of straw inoculations a clean mung bean agar plug was used. For the second experiment, only the cotton wool method was used.

Field trials:

The *F. graminearum* isolates used for these trials were obtained from the University of the Free State and originated from FHB in the Prieska region. The twenty isolates used for spray inoculum for both 2007 and 2008 trials were A144, A145, A146, A149, A151, A156, A159, A161, A166, A167, A169, A176, A183, A187, A191, A194, A204 A221, A223 and A269.

Thirty mung bean agar Petri dishes were prepared for each isolate and incubated at 22℃ for four weeks before harvesting. Each Petri dish was

blended with 50 ml of distilled water. Both trials were inoculated during anthesis at GS 59 and 60. During the 2008 trial, spray inoculum was combined with grain spawn (Steffenson, 2003; Verges *et al*., 2006).

The grain spawn was prepared on overnight soaked maize kernels placed in autoclave bags and sterilised on consecutive days for 30 min. Each bag was inoculated with one isolate. A cotton wool plug was used for aeration and the grain was incubated for four weeks at 23℃. Colonized grain spawn was dried and scattered in the field, three weeks before flowering. The advantage of grain spawn inoculum is the generation of ascospore inoculum over a period of time, rather than when inoculum is sprayed. It is also useful where access to areas is limited for spraying inoculum and it is easy to prepare. Grain spawn can also be used in combination with spray applications (Dill-Macky, 2003; Gilbert & Woods, 2006).

The 2007 and 2008 trials were inoculated four times at two-day intervals, by spraying with a Stihl® mist blower (Fig. 3.3) at GS 59. The inoculation dates for 2007 was between 31 October 2007 to 7 November 2007 and in 2008 between 27 October 2008 and 3 November 2008, respectively.

Figure 3.3: Spray inoculations executed in the field under floppy irrigation at Bethlehem.

The mist blower was calibrated beforehand. To spray the honeycomb block of 45.36 m^2, a total of 6.7 liter of inoculum was applied on all honeycomb entries. Total spraying time was determined to be 2 min and 20 sec for the trial. Maize inoculum was scattered in the field at GS 32 (30 September 2008). Evaluations started two weeks after the first inoculations.

3.2.1.4 Evaluation of the disease

In both greenhouse trials, disease symptoms were evaluated at 7, 10, 14, 17 and 21 days after inoculation. In both the greenhouse and field trials, disease incidence/severity was recorded as the number of bleached florets in relation to the total number of florets and expressed as a percentage. Greenhouse evaluations measured Type II resistance whereas Type I resistance was measured in the field.

3.2.1.5 Data handling and statistical analysis

Greenhouse trials:

The experiment was set up as a randomized complete block design using one isolate, three inoculation methods and a control, with four replications in each treatment. Each replication consisted of twenty individual heads.

The AUDPC was calculated with the equation described below (Shaner & Finney, 1977; Campbell & Madden, 1990; Buersmayr *et al*., 2000; Jeger & Viljanen-Rollinson, 2001).

$$\text{AUDPC} = \sum_{i}^{n-1} \{[(y_i - y^i_{+1})/2] \ (t_{i+1} - t_i)\}$$

(n = the total number of observations; y^i = visually infected spikelets on the *i*-th day; t_i = day of the *i*-th observation).

ANOVA was performed with GenStat® Release 10 (Payne, 2007), to determine the:

- statistical differences between the inoculation methods or entries,

- least significant differences (LSD), and
- coefficient of variance (CV).

The LSD significance level depended on the heterogeneity of variances.

Field trials:

A honeycomb design, normally used to evaluate progenies from wheat crosses, was used in both years. This design in particular overcomes interplant competition and soil heterogeneity. Entries are evaluated by moving the grid of the honeycomb. This moving grid secures control of environmental variation as similar distances and constant criteria of evaluation are ensured. Yield is also compared to the equidistantly spaced surrounding neighbours (Mitchell *et al*., 1982; Fasoulas & Zaragotas, 1990).

This design is effective, inexpensive, uses limited space and there are almost no limits to the amount of material that can be evaluated. A limited amount of seed can be used (Lungu *et al*., 1987; Fasoulas, 1988). In these studies the entries were evaluated as a group and compared against the resistant and susceptible controls.

Analysis of the 2007 and 2008 field trials were done using Microsoft Office Excel 2003. The reactions of the four moderate resistant and four susceptible entries surrounding the 9th SRSN entry are determined separately. A mean for each of the resistant and susceptible entries was determined. The susceptible mean is divided by the mean for the resistant entries, providing an infection value. The actual percentage allocated to the 9th SRSN entry was divided by the resistance reaction to give a resistance value. To determine the percentage of infection, the actual percentage was divided by the infection value and multiplied by 100. The entries are then divided into groups ranging from resistant to very susceptible according to the scale used by CIMMYT, Mexico, where the percentage of infection was linked to the response of the entries.

3.2.2 RESULTS AND DISCUSSION

3.2.2.1 Experiment 1: Greenhouse trial

The different inoculation techniques were tested in the greenhouse over a period of four weeks.

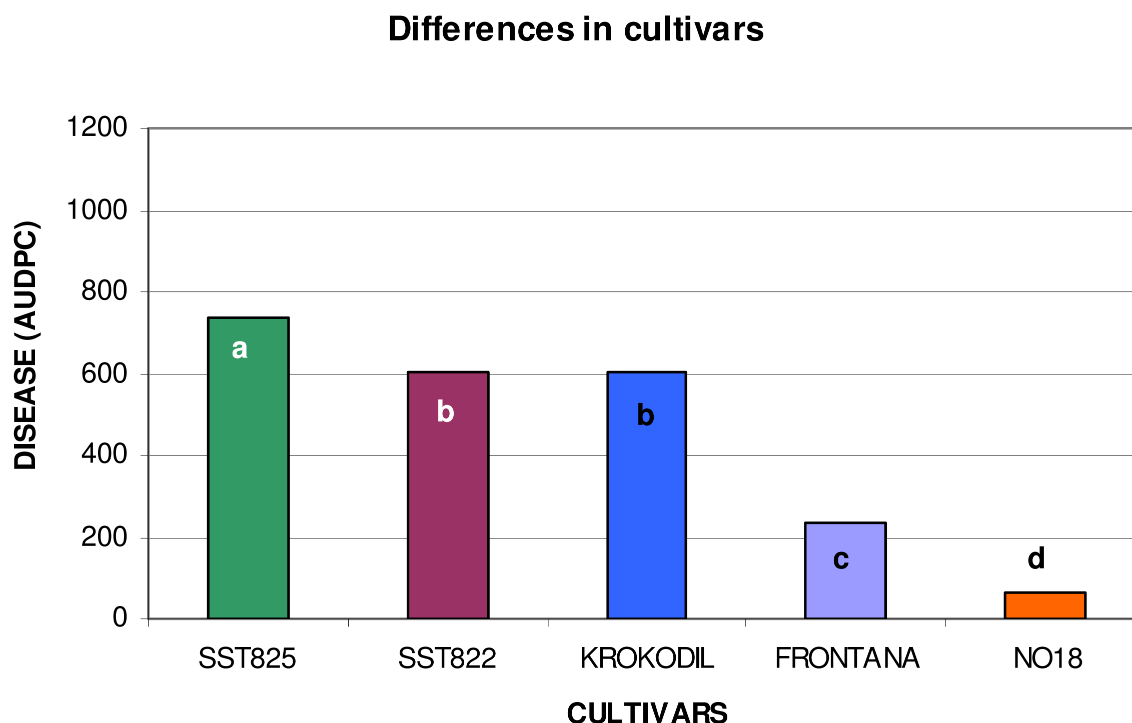

Figure 3.4: Ranking of the cultivars over all the inoculation techniques tested.

According to the AUDPC (Fig. 3.4) SST825 was the most susceptible wheat cultivar to FHB. SST822 and Krokodil did not differ significantly from each other and Frontana and no. 18 were the most resistant. According to Kriel and Pretorius (2008) as well as Anonymous (2005), SST825 is highly susceptible, and SST822 and Krokodil are moderately susceptible to FHB. According to literature reports Frontana and Sumai #3 are of the most resistant cultivars against FHB and are widely used in wheat hybridization (Bai *et al.*, 2003; Mesterházy, 2003). No. 18 is a cross between Sumai #3 and Yangmai 158, where Yangmai 158 was used to widen the genetic base

for scab resistance (McKendry, 2000). The LSD (1%) was 63.03 and the CV was 12.8%.

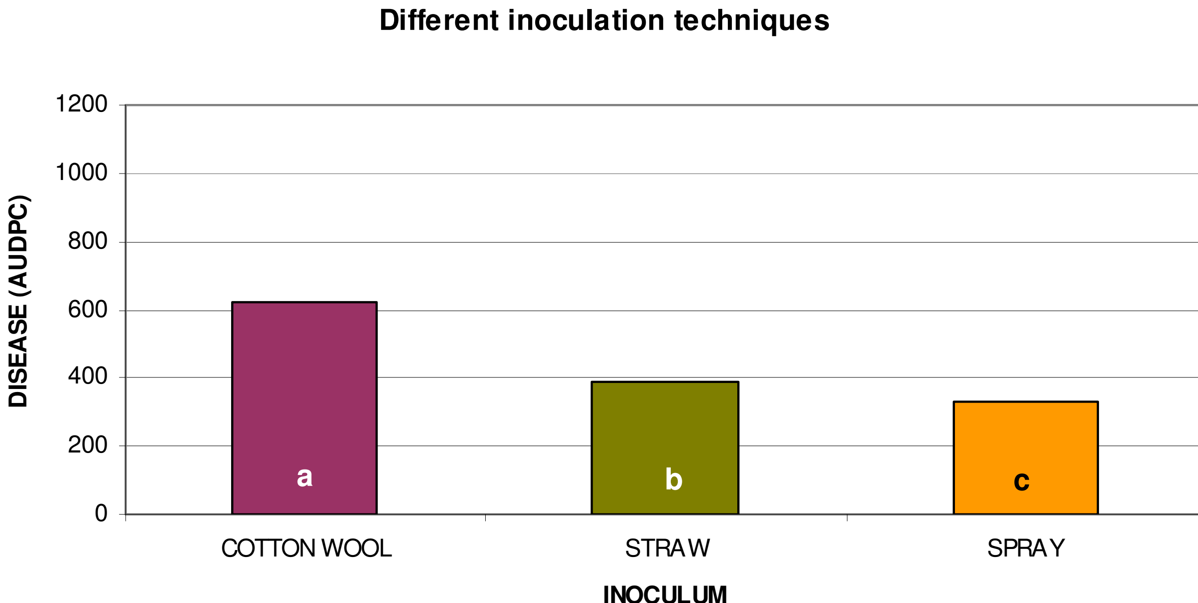

Figure 3.5: Inoculation techniques tested over five cultivars to determine the best inoculation method for the greenhouse.

The data for the inoculation techniques (Fig. 3.5) showed that the cotton wool method was the best procedure for testing Type II resistance in a greenhouse, which corresponds with other reports (Van Ginkel *et al.*, 1996; Ntushelo & Boshoff, 2002; Bushnell *et al.*, 2003; Steffenson, 2003). Bekele (1984) also stated that this is a more precise method in controlled studies. The spray inoculation was used to determine Type I resistance. The LSD (1%) for the inoculation methods was 48.83 and the CV 12.8%. The effect of inoculation techniques on the different cultivars can be seen in Fig. 3.6.

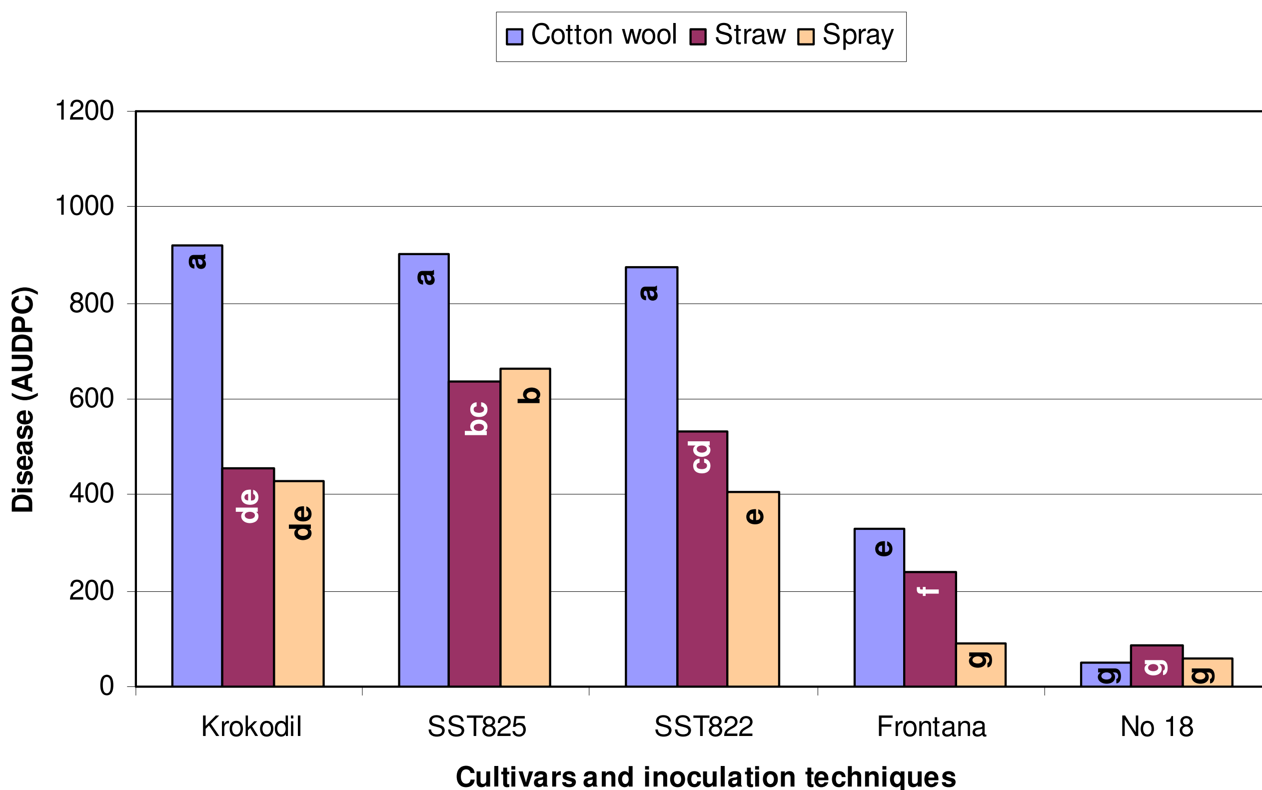

Figure 3.6: Different inoculation techniques between different cultivars in the greenhouse.

Krokodil cotton wool (CW), SST825 CW and SST822 CW did not differ significantly from each other (Fig. 3.6). SST825 spray (SPR) and SST825 straw (STR) did not differ significantly from each other, although SST825 SPR did differ significantly from SST822 STR and the rest of the entries. Frontana and no. 18 had the lowest AUDPC indicating the resistance levels in these cultivars. Sumai #3 is commonly known to have Type II resistance (Bai & Shaner, 1994; Fedak *et al*., 2007) but also has Type I resistance. According to McKendry (2000), Bushnell *et al*. (2003) and Shaner (2003), Sumai #3 has a high combining ability and has been widely used as a parent in breeding programmes worldwide.

The cotton wool and straw inoculation methods did not differ between the susceptible cultivars. SST825 SPR had a significantly higher AUDPC than the rest of the spray inoculations as this cultivar is more susceptible to the pathogen. The inoculation techniques differed significantly for Frontana

but not for no. 18. Frontana, which originated in Brazil, has the pedigree 'Fronteira'/'Mentana' (Jiang *et al.*, 2006). No. 18 is a cross, between Sumai #3 and Yangmai 158 (Attachment C). Sumai #3 was developed at the Suzhou Institute of Agricultural Science in Jiangsu Province, China. The resistance in Sumai #3 is characterized by high levels of stable resistance, low incidence, reduced spread and low toxin levels in grain colonized by the pathogen. These are the main reasons why Sumai #3 is used extensively in breeding programmes over the world (McKendry, 2000; Bai *et al.*, 2003; Jiang *et al.*, 2006; Jiang *et al.*, 2007). Yangmai 158 is moderately resistant to Fusarium head blight and is a high yielding variety (http://www.cimmyt.org/english/docs/book/historywbchina/pdf/HistWBChina_cap4.pdf). The LSD (1%) for the interaction between the inoculation methods and the cultivars was 109.18 and the CV 12.8%.

Spray inoculation is currently the only inoculation method to determine Type I resistance (Schroeder & Christensen, 1963; Mesterházy, 2003; Yu, 2007). The advantage of inoculation with cotton wool is described in Chapter 2. Although the straw inoculation method is also effective, the disadvantage of this method is that a wheat floret is injured during inoculation. An advantage of this method is that the plants are not placed in a dew chamber.

3.2.2.2 Experiment 2: Germplasm evaluation

Twenty lines from the 9th SRSN were used in this study and included nos. 3, 5, 14, 19, 21, 28, 31, 34, 38, 39, 42, 47, 50, 52, 54, 57, 58, 61, 62 and 74. Lines 28, 31, 38, 42, 52, 58, 62 did not emerge or did not produce heads for inoculation. SST825 and Sumai #3 were the susceptible and resistant controls respectively. Lines/cultivars were evaluated over a four week period (Fig. 3.7).

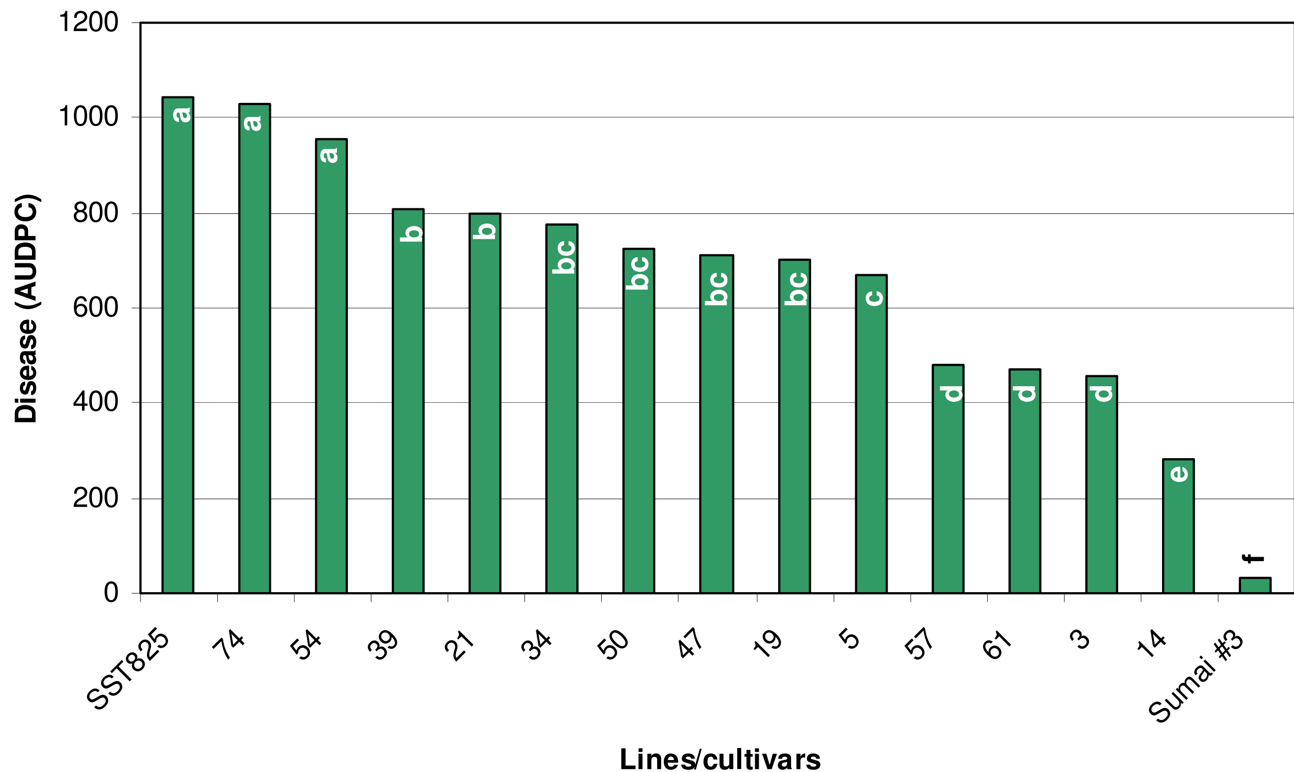

Figure 3.7: Disease progress in lines/cultivars tested.

SST825 had the highest FHB AUDPC and Sumai #3 had the lowest. This was to be expected as SST825 is susceptible and Sumai #3 resistant to FHB (Anonymous, 2005; Nicholson *et al*., 2007; Ban *et al*., 2008; Kriel & Pretorius, 2008). No significant differences occurred between SST825, nos. 74 and 54, showing that these two lines were as susceptible as SST825. Lines nos. 39, 21, 34, 50, 47, 19 and 5 were considered moderately susceptible. However, there are significant differences between this group and nos. 57, 61 and 3, and no. 14, which were moderately resistant. Sumai #3 was resistant. The LSD at 5% confidence level was 121.7 and a CV of 7.1%. From this trial it is evident that nos. 57, 61, 3 and 14 were more resistant to FHB. In this trial only type II resistance was determined and these lines may thus be utilised in breeding where factors such as adaptation, yield and quality are taken into consideration.

3.2.2.3 Experiment 3: Field trials

FHB was first noted two weeks after inoculation and was assessed as a percentage of spike infection. According to their FHB responses, the 9th SRSN entries were grouped from resistant (R) to very susceptible (VS) (Table 3.1).

Table 3.1: A modified Japanese scale for evaluation of scab in the field (Ireta & Gilchrist, 1994).

SCALE	PERCENTAGE INFECTION (SPIKES OR WITHIN SPIKELETS)	LEVELS OF RESPONSE
1	1 – 5%	R
2	5 - 25%	MR
3	25 - 50%	MS
4	50 - 75%	S
5	> 75%	VS

Note: R = Resistant; MR = Moderate resistant; MS = Moderate susceptible; S = Susceptible; VS = Very susceptible

The percentage total blighted spikes (incidence), rating and yield of both 2007 and 2008 field trials are shown in Table 3.2.

Table 3.2: Mean values of blighted head percentage and rating as well as total yield for the inoculated wheat entries in the field for 2007 and 2008 to determine Type I resistance.

9th SRSN ENTRY NUMBER	*BLIGHTED HEAD % (2007)	RATING (2007)	TOTAL YIELD (g) (2007)	*BLIGHTED HEAD % (2008)	RATING (2008)	TOTAL YIELD (g) (2008)
1	**	**	**	0.50	R	6.12
2	0.69	R	45.13	0.56	R	11.65
3	8.24	MR	57.78	0.21	R	5.15
4	43.17	MS	26.12	0.48	R	9.8
5	4.23	R	57.29	1.69	R	7.64
6	11.76	MR	46.5	0.34	R	5.63
7	5.93	MR	36.33	6.36	R	6.43
8	1.78	R	26.93	0.45	R	1.7
9	0.88	R	53.54	0.37	R	11.46
10	3.79	R	14.22	1.63	R	19.16
11	28.64	MS	15.48	1.92	R	12.76
12	1.02	R	74.64	0.64	R	23.99
13	30.00	MS	30.74	0.66	R	10.26
14	2.19	R	83.28	2.81	R	10.11
15	2.65	R	76.18	1.20	R	22.15
16	8.44	MR	56.13	0.90	R	8.46
17	0.29	R	18.64	0.47	R	5.6
18	2.83	R	51.45	0.63	R	10.56
19	5.38	MR	59.95	2.57	R	5.25
20	3.61	R	70.42	0.61	R	19.55
21	1.44	R	67.18	3.73	R	2.94
22	20.11	MR	33.78	0.24	R	11.79
23	13.64	MR	21.12	3.88	R	4.58
24	2.34	R	49.87	**	**	**
25	**	**	**	0.77	R	12.95
26	17.50	MR	38.85	0.46	R	8.71
27	15.64	MR	76.54	1.90	R	2.09

Table 3.2: Mean values of blighted spike percentage, rating and yield for the inoculated wheat entries in the field for 2007 and 2008 cont'

9th SRSN ENTRY NUMBER	*BLIGHTED HEAD % FOR 2007	RATING FOR 2007	TOTAL YIELD (g) FOR 2007	*BLIGHTED HEAD % FOR 2008	RATING FOR 2008	TOTAL YIELD (g) FOR 2008
28	5.17	MR	57.41	3.10	R	13.34
29	22.13	MR	63.08	0.33	R	15.31
30	1.58	R	53.18	0.60	R	4.86
31	3.00	R	49.48	0.12	R	11.27
32	28.42	MS	50.94	0.17	R	1.68
33	6.03	MR	73.49	0.30	R	4.88
34	5.63	MR	43.94	2.64	R	9.56
35	24.63	MR	17.88	2.80	R	14.11
36	0.58	R	21.78	**	**	**
37	2.19	R	47.4	0.16	R	5.31
38	4.43	R	74.48	1.09	R	5
39	13.08	MR	53.48	2.06	R	3.07
40	11.21	MR	25.98	0.45	R	14.45
41	16.38	MR	50.23	2.14	R	6.27
42	8.94	MR	28.06	6.29	R	11.14
43	2.87	R	42.97	0.80	R	18.51
44	2.39	R	51	0.55	R	7.7
45	33.00	MS	28.33	3.31	R	7.23
46	11.89	MR	47.66	3.90	R	20.54
47	0.41	R	29.85	**	**	**
50	10.69	MR	53.68	0.86	R	12.43
51	0.37	R	118.73	0.37	R	5
52	5.71	MR	67.16	0.21	R	7.04
53	0.92	R	71.12	1.12	R	5.79
54	11.19	MR	66.63	7.96	R	0.82
55	1.69	R	21.8	0.30	R	7.22
56	1.14	R	56.22	0.44	R	8.58
57	5.26	MR	30.7	4.35	R	3.15
58	8.30	MR	40.78	1.64	R	7.06

Table 3.2: Mean values of blighted spike percentage, rating and yield for the inoculated wheat entries in the field for 2007 and 2008 cont'

9th SRSN ENTRY NUMBER	*BLIGHTED HEAD % FOR 2007	RATING FOR 2007	TOTAL YIELD (g) FOR 2007	*BLIGHTED HEAD % FOR 2008	RATING FOR 2008	TOTAL YIELD (g) FOR 2008
59	0.45	R	46.89	0.29	R	17.64
60	0.51	R	74.91	0.33	R	6.72
61	7.79	MR	80.33	1.00	R	6.09
62	3.70	R	42.95	1.69	R	4.59
63	22.50	MR	28.33	0.17	R	5.97
64	22.88	MR	49.13	2.72	R	10.86
65	2.56	R	40.85	3.64	R	1.39
66	50.49	S	45.33	0.46	R	19.87
67	1.58	R	58.48	1.69	R	3.38
68	5.89	MR	21.27	0.57	R	6.09
69	**	**	**	0.34	R	0.87
70	8.24	MR	55.7	1.35	R	1.65
71	2.53	R	40	0.45	R	14.63
72	0.62	R	30.06	0.14	R	4.03
73	0.34	R	13.2	0.83	R	1.42
74	7.12	MR	52	0.92	R	15.55
75	**	**	**	0.40	R	6.51
SST825	24.23	MR	59.95	12.39	R	17.64
Sumai #3	0.83	R	60.92	0.71	R	9.37

Note: * Total value of honeycomb calculations.
** 24, 25, 36, 47, 48, 49, 69 and 75 did not emerge and number 1 did not produce heads for inoculations and evaluation.
R = Resistant,
MR = Moderate resistant,
MS = Moderate susceptible,
S = Susceptible, and
VS = Very Susceptible.

Data from 2007 showed only one entry that was susceptible, namely no 66. There are 33 lines that showed resistance, 29 that showed moderate

resistance and five that showed moderate susceptibility. No very susceptible cultivars were recorded, but one was rated in the susceptible category. During the season, the weather was conducive to promote FHB infection. During the flowering stage when the wheat was inoculated, the mean RH during the night was at 92% and 52% during day time. Rainfall was regular during this period and the trial was irrigated when needed. The ETO throughout the season was very low and was reflected in the temperatures that were on average 23℃ during flowering (Fig. 3.8 & Fig. 3.9). Environmental conditions such as RH and temperatures between 25℃ and 30℃ are conducive to FHB (Wiese, 1987) with a lower limit at 15℃ (Sutton, 1982). High RH for between 12 – 15 h as well as frequent rainfall will be sufficient for an epidemic (Shaner, 2003).

According to 2007 data, it is clear that most of the entries for 2008 should have been resistant, but due to the environmental conditions such as RH and rainfall, low infection levels were achieved. During this time, the weather was not conducive to promote FHB infection in the field. During flowering stage, when the wheat was artificially inoculated, the mean RH during the night was very high at 91%, but the mean during the day was very low (25%). Rainfall was irregular during this time and the ETO throughout the season was higher than in the previous year (Fig. 3.10 & Fig. 3.11). According to Beyer & Verreet (2005) a small percentage of ascospores will germinate at 53% RH at 20℃ and the percentage of viable spores decreased to 6% within 10 min. This means that ascospores need favourable conditions such as RH for germination immediately after discharge. A misting system in the field will also promote FHB infection by increasing the RH in the field (Wilcoxson *et al.*, 1992; Parry *et al.*, 1995).

If 2008 data are compared with the previous year, it is clear that not all the entries were resistant. Even though extra inoculum was available in the form of colonized grain, the disease did not develop as it should have. According to Anderson (1948) the inoculum dosage, period of exposure of the inoculated plants to moisture, the air temperature at the time of inoculation and the growth stage when the plants are inoculated, all have an influence on disease development. The period of exposure to moisture that is necessary for infection becomes progressively longer at temperatures that are higher or lower than 25℃. High levels of moisture are also necessary where colonized grain is used to promote disease development (Sutton, 1982; Wilcoxson *et al.*

1992). According to trials that were executed by Wilcoxson *et al*. (1992) during 1987 and 1988, FHB did not develop satisfactorily despite frequent sprinkling to promote disease development. Mesterházy (1997) indicated that two to three year data are necessary if field evaluations are to be regarded with confidence by ruling out environmental variation.

3.2.2.4 Weather data for field trials

For the 2007 and 2008 weather data, the dates that were included in the graph are from two weeks before flowering, up until harvest. Data is shown for the minimum and maximum temperatures (Fig. 3.8, Fig. 3.9, Fig. 3.10 & Fig. 3.11). All data was obtained from http://155.240.219.9/StationSearch_ByName/ByName__GetDa tes.php

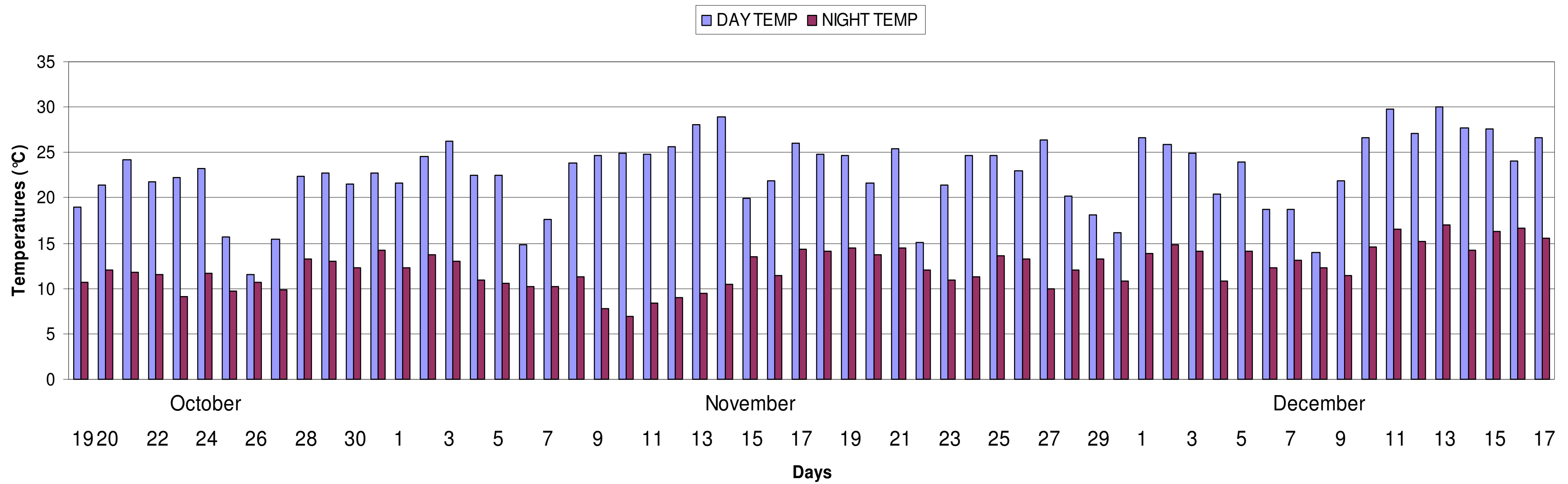

Figure 3.8: Temperatures from October to December 2007 under the floppy irrigation system at SGI, Bethlehem.

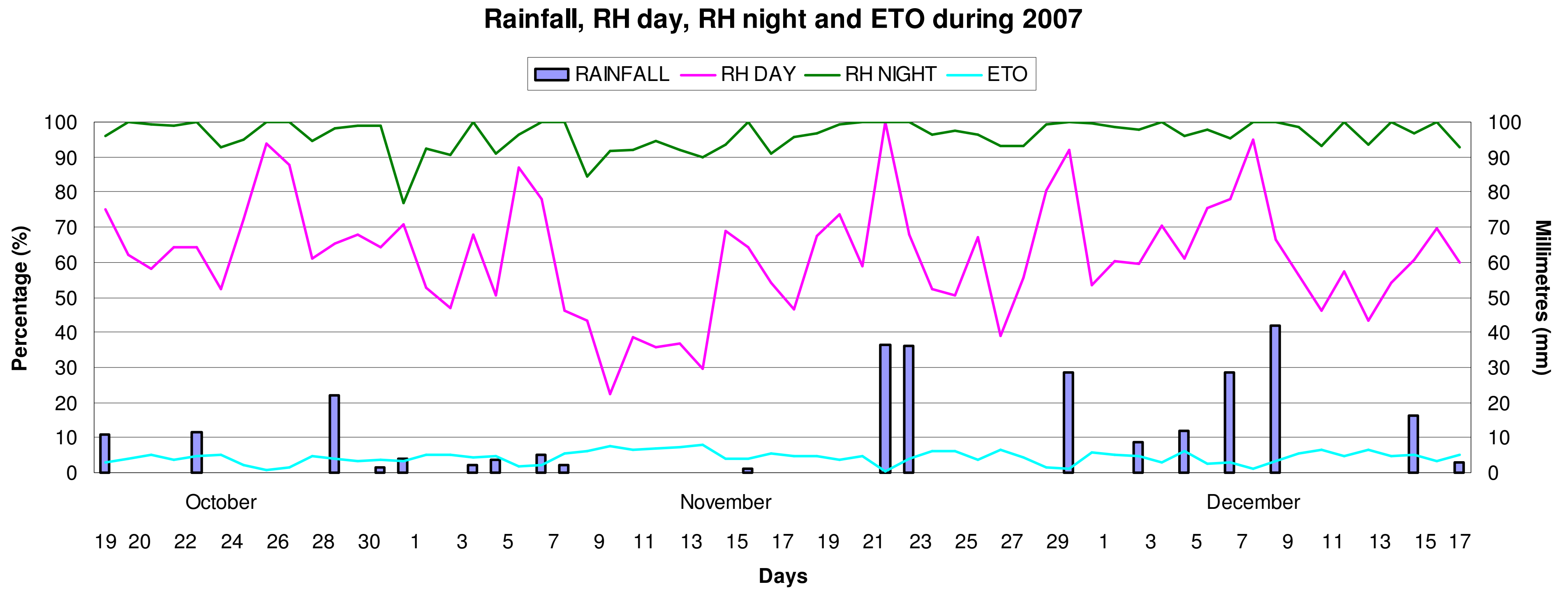

Figure 3.9: Rainfall, RH day, RH night and ETO during 2007.

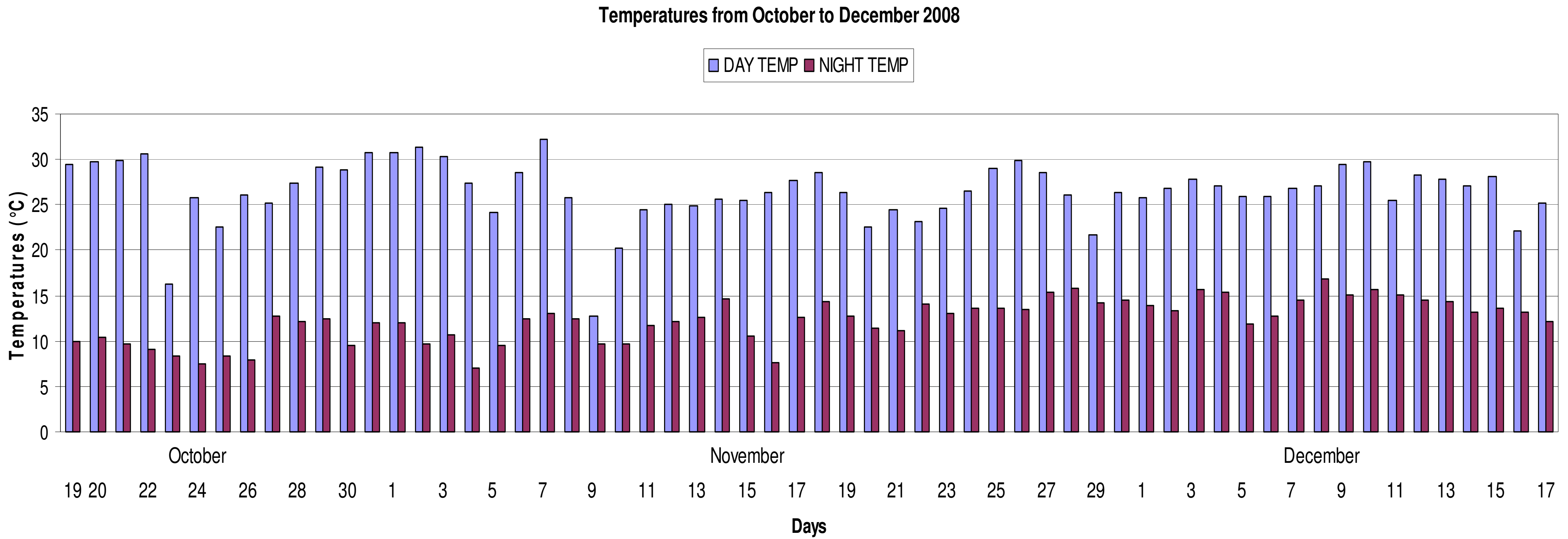

Figure 3.10: Temperatures from October to December 2008 under the floppy irrigation system at SGI, Bethlehem.

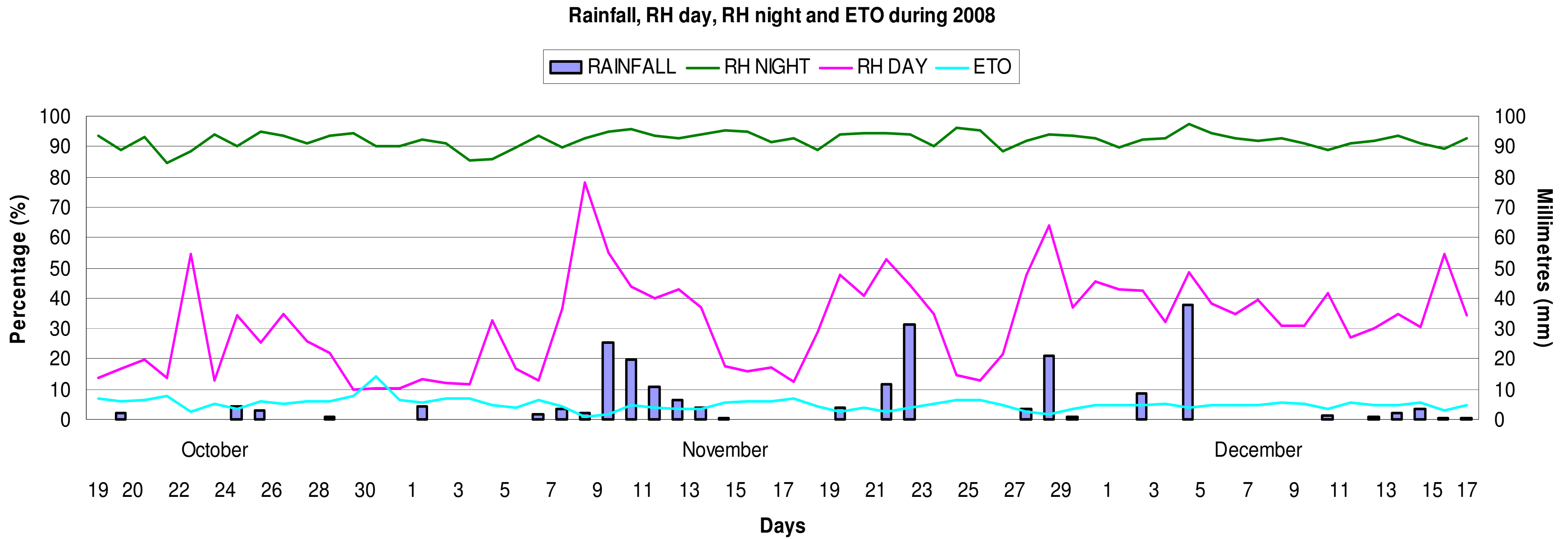

Figure 3.11: Rainfall, RH day, RH night and ETO during 2008.

Screening techniques for FHB resistance should be improved in order to rapidly select resistant sources. By using the honeycomb moving grid selection for the field trials, only a small amount of seed is needed. The absence of interplant competition between plants is beneficial and the small field size is advantageous (Lungu *et al*., 1987; Fasoulas, 1988). However, in the present study the honeycomb design did not allow a meaningful comparison of the relationship between disease levels and yield. In both years the percentage head blight did not explain the variation in yield (2007: R^2 = 0.08; 2008: R^2 = 0.026). Thus, more experimentation is needed to develop field procedures for correlating FHB and yield.

Inoculation methods such as maize inoculum or spraying plots are easy and simple techniques to execute. These methods also simulate natural situation conditions more closely. However, the infection processes can vary, depending on the environmental conditions, soil type and the viability of isolates used. The grain spawn method can be used in mass screening in the field to determine resistance in all available material, although these lines should be screened again to verify resistance in materials (Bai *et al*., 2003; Dill-Macky, 2003). Field evaluations can be conducted once a year and therefore breeding programmes rely on greenhouse procedures to achieve progress towards the development of resistant germplasm (Engle *et al.,* 2003).

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Annexure A

1. CARROT AGAR (CA; Dhingra & Sinclair, 1985)

Materials:

Sliced carrots	20 g
Agar agar (Biolab)	19 g
Distilled water	1 litre

Method:

Peeled carrots were cooked in one litre of distilled water until soft. The carrots were sieved through cheesecloth, adjusted to one litre and 19 g agar agar (Biolab) was added. The CA mixture was autoclaved, cooled and 30 ml of media were poured in 90 mm, plastic, disposable, sterile Petri dishes.

2. POTATO DEXTROSE AGAR (PDA; Jones & Clifford, 1983)

Materials:

PDA (Biolab)	39 g
Distilled water	1 litre

Method:

Potato dextrose agar (Biolab) was added to one litre of distilled water, autoclaved, cooled and poured as described above.

3. MUNG BEAN AGAR (MBA; Gilchrist-Saavedra *et al.*, 1997; e-mail communication: Dr Ruth Dill-Macky)

Materials:

Mung beans	20 g
Agar Agar (Biolab)	15 g
Distilled water	1 litre

Method:

Mung beans were cooked for 23 minutes in one litre of distilled water. The pericarp of the mung beans must start to burst open before taking it off the heat. The broth is sieved through two layers of cheesecloth and is adjusted to one litre broth again. Fifteen g agar agar (Biolab) is then added to the mung bean broth and autoclaved for 15 minutes at 121℃. The medium were cooled down to 45℃ and then poured into sterile Petri dishes as described above.

4. MALT EXTRACT AGAR (MEA; Gilchrist-Saavedra *et al.*, 1997)

Materials:

Malt Extract (Biolab)	20 g
Agar Agar (Biolab)	19 g
Shredded dry maize material (weighed and autoclaved before adding to MEA)	5 g

Method:

Malt extract (Biolab) and agar agar (Biolab) were added together with 5 g of dry, sterile, shredded maize material. This mixture was autoclaved, cooled and poured as described above.

Annexure B

Entries from the 10th SRSN, CIMMYT, Mexico used in the field and greenhouse trials.

Entry	Cross Name
1	TUI/MILAN
2	HEILO
3	NG8675/CBRD//MILAN/3/NG8675/CBRD
4	NING MAI 9558
5	RECURRENT SELECTION 1
6	CATBIRD
7	CS/TH.CU//GLEN/3/GEN/4/ALD/PVN/5/SUZ8
8	MILAN/SHA7
9	TINAMOU
10	TRAP#1/BOW//TAIGU DERIVATIVE
11	TRAP#1/BOW//TAIGU DERIVATIVE
12	SHA3/CBRD
13	SHANGHAI
14	SUM3/3/CS/LE.RA//CS/4/YANGMAI 158
15	PI/MN72131
16	PRINIA
17	SHA4/CHIL
18	SUM3/3/CS/LE.RA//CS/4/YANGMAI 158
19	WUH1/VEE#5//CBRD
20	YANGMAI 5
21	SHANGHAI
22	CBRD//KPOPO/MBU
23	CHIL/CHUM18
24	GONDO
25	GONDO
26	MILAN/CEP8953//PF869120
27	NG8675/CBRD
28	PEL 73007
29	SHA3/SERI//SHA4/LIRA

Entry	Cross Name
30	SITELLA
31	BR23/EMB27
32	RUSS/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/RABI/5/ AE.SQUARROSA (878)
33	TRAP#1/BOW//TAIGU DERIVATIVE
34	SHA3/CBRD
35	EMB27/KLORI
36	IVAN/6/SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)
37	BR23/EMB27

Annexure C

Entries from the 9th SRSN, CIMMYT, Mexico used in the field and greenhouse trials.

Entry	Cross Name
1	605-87/SNB
2	80456/YANGMAI 5
3	ALBERT/FDRC
4	ATTILA/5/FURY-KEN/SLM//ALDAN/4/PAT10/ALD//PAT72300/3/PVN/6/DUCULA
5	BAU/MILAN//CBRD
6	BAU/MILAN//CBRD
7	BAU/MILAN//CBRD
8	BAU/MILAN//CBRD
9	BAU/MILAN//CBRD
10	BCHA/MILAN
11	BJY/COC//PRL/BOW
12	BNDU/CONA/3/9.72/BNAP//COCA
13	BOW//BUC/BUL/3/KAUZ/4/CHOIX
14	BPAL/3/COCA/BCEN//BNAM
15	BR 23
16	CATBIRD
17	CATBIRD
18	CHAT/CEP7780//PRL/BOW
19	CHIL/CHUM18
20	CHIL/IAN8
21	CHIL/URES
22	CNO79/IAC24
23	COOPERACION MAIPUN
24	ESTANZUELA COLIBRI
25	EHAL//CHUM18/BAU
26	EHAL//CHUM18/BAU
27	EMB27/CEP8825//MILAN
28	EMB27/CEP8825//MILAN
29	FILIN/MILAN

Entry	Cross Name
30	GONDO
31	GONDO//BAU/MILAN
32	GRANERO INTA
33	ESDA/WEAVER
34	HEILO
35	IAN 8-PIRAPO
36	INIA CABURE/INIA TIJERETA
37	INIA CABURE/LAJ3153
38	IRENA/CETTIA/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL
39	ITAPUA 45 - DON PANI
40	ITAPUA 50 - AMISTAD
41	KLCAR/SNB
42	KAKATSI
43	KAUZ//TRAP#1/BOW
44	KAUZ/4/GOV/AZ//MUS/3/KEA
45	LFN/II58.57//PRL/3/HAHN
46	MILAN/AMSEL//CBRD
47	MILAN/AMSEL//CBRD
48	MILAN/SHA7
49	MON/IMU//ALD/PVN
50	MURGA
51	PROINTA ALAZAN
52	PF85235/SA8615/5/CEP8879/4/KLAT/SOREN//PSN/3/BOW
53	PF8944/BBGL//BR23/EMB27
54	PGO/SARA
55	PRINIA
56	PROINTA GRANAR
57	R37/GHL121//KAL/BB/3/JUP/MUS/4/2*YMI #6/5/CBRD
58	SHA3/SERI//SHA4/LIRA
59	SHA3/SERI//YANG87-142
60	SHA4/3/2*CHUM18//JUP/BJY
61	SURUTU-CIAT

Entry	Cross Name
62	SW89.5277/BORL95//SKAUZ
63	SW89-5124*2/FASAN
64	SW89-5124*2/FASAN
65	SWM7079/KLSL//KLCH
66	TAM200/TUI
67	THB/KEA//PF85487/3/MILAN
68	THB/KEA//PF85487/3/RIVADENEIRA 4
69	THB/KEA//PF85487/3/RIVADENEIRA 4
70	TNMU//LIRA/VEE#7
71	TNMU//LIRA/VEE#7
72	TRAP#1/BOW//TAIGU DERIVATIVE
73	TRAP#1/BOW//TAIGU DERIVATIVE
74	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC
75	WUHAN #3

SUMMARY

Fusarium head blight is one of the most important wheat diseases under irrigation and currently there are no cultivars resistant to this disease in South Africa. The main aim of this study was to determine the virulence of *Fusarium graminearum* isolates, to test growth and sporulation on different media and to do screening for resistance in the greenhouse and field.

Twenty *Fusarium graminearum* single spore isolates from the Prieska region were evaluated in the greenhouse to determine the virulence of the isolates on a susceptible wheat cultivar. The isolates that were most virulent were A191, A145, A144, A149, A204 and A223. These isolates did not differ significantly from each other in the greenhouse test. Five of these isolates were used in the laboratory to determine which solid medium can be used to produce an abundance of spores. Potato dextrose agar, carrot agar, malt extract agar with 5 g chopped maize straw and mung bean agar were used. The growth of the isolates was measured over a seven day period. From the data it was evident that malt extract agar and mung bean agar were the best media to use for growth within the seven day period. Each of the media was tested weekly for up to four weeks to determine which of the isolates produced the most spores when given optimum conditions. Mung bean agar was the best medium to use for spore production. Considering the other media, potato dextrose agar may be used for identification, multiplying and storage of *Fusarium* isolates. Carrot agar is suitable to induce spore production, but is generally used as a medium for fertility studies. Malt extract agar containing sterile, chopped maize straw may be used for growth rather than for inducing spore formation. A lower amount of malt extract should be used to reduce the amount of maltose in the medium to increase spore production.

Isolate A191 was used in the other two greenhouse tests. The first test was to determine the best inoculation technique for inducing FHB. Five different cultivars were included, namely SST825, SST822, Krokodil, Frontana and a derivative of Sumai #3 (SUM3/3/CS/LE.RA//CS/4/YANGMAI 158). Three techniques were used, namely cotton wool, straw and spray inoculation. From the data obtained it was clear that the cotton wool method was the best inoculation method to use to test Type II resistance. In a second greenhouse test, 13 lines from the 9th SRSN, CIMMYT, Mexico, were tested with isolate A191 using the cotton wool technique. The results showed that there were no differences between the moderately susceptible SST825 and numbers 74 and 54 (Type II resistance). The second group can be classified

as a moderately resistant group that included numbers 54, 39, 21, 34, 50, 47, 19, 5, 57, 61 and 3. Number 14 was classified as resistant and the Sumai #3 derivative as a very resistant cultivar.

Two field trials were executed in 2007 and 2008, respectively. The 9th SRSN from CIMMYT, Mexico, were included in the trials for both years. The field experiment consisted of the honeycomb moving grid design. The isolates used for both years included numbers were A144, A145, A146, A149, A151, A156, A159, A161, A166, A167, A169, A176, A183, A187, A191, A194, A204 A221, A223 and A269. These isolates were prepared on mung bean medium, harvested and sprayed four times, at two-day intervals, onto wheat plants during flowering. The environmental conditions for the 2007 trial were excellent for infection. Data from 2007 showed that entry 66 was susceptible. Thirty-three lines showed resistance, 29 lines showed moderate resistance and five showed moderate susceptibility. SST806 and Marico were used as a susceptible and moderate resistant control, respectively.

The environmental conditions for 2008 were not conducive and very low levels of infection were obtained. Grain spawn was used in conjunction with spray inoculation to improve the inoculum pressure during 2008. Although extra inoculum was added, a low infection percentage was noted. Thus, field trials should be executed for at least three seasons before meaningful recommendations can be made.

OPSOMMING

Fusarium aarskroei is een van die mees belangrikste siektes van koring onder besproeiing en tans is daar geen weerstandbiedende kultivar teen aarskroei in Suid-Afrika nie. Die hoofdoel van die studie was om die virulensie van twintig *Fusarium graminearum* isolate te bepaal, om die virulensie op verskeie media te toets en om inskrywings in die glashuis en veld vir weerstand te evalueer.

Twintig *Fusarium graminearum* enkelspoor isolate vanaf die Prieska omgewing, is in die glashuis geëvalueer om die virulensie van die isolate te bepaal. Die isolate wat die beste presteer het in die glashuis was A191, A145, A144, A149, A204 en A223. Hierdie isolate het nie betekenisvol van mekaar in die glashuisevaluasie verskil nie. Vyf van die isolate is in die laboratorium toets gebruik om vas te stel watter soliede medium die meeste spore sal vorm. Aartappel dekstrose-agar, wortel-agar, mout ekstrak-agar met 5 g opgekapte mieliestoppel en mung boontjie-agar is gebruik. Die groei van die isolate is gemeet oor 'n sewe dae tydperk. Die data het getoon dat mout ekstrak-agar en mung boontjie-agar die beste media vir groei, was. Elkeen van die media is weekliks getoets, oor 'n tydperk van vier weke, om die isolaat wat die meeste spore onder gunstige toestande produseer, te bepaal. Die data toon aan dat die mung boontjie-agar die beste medium is om te gebruik vir spoorproduksie. Die afleiding kan gemaak word dat aartappel dekstrose-agar gebruik kan word vir identifikasie, vermeerdering en stoor van *Fusarium* isolate. Wortel-agar kan gebruik word vir spoorproduksie, alhoewel dit ook gebruik kan word vir vrugbaarheidstudies. Mout ekstrak-agar saam met die opgekapte mieliestoppel sal eerder gebruik word vir groei van die isolate en vir die produksie van groot hoeveelhede spore. Die hoeveelheid mout ekstrak moet verlaag word in verdere studies ten einde die hoeveelheid maltose in die medium te verminder wat die moontlikheid van spoorproduksie sal verhoog.

Isolaat A191, is gebruik vir twee ander glashuistoetse. Die eerste glashuistoets was om die beste inokulasie metode vas te stel. Vyf verskillende cultivars is gebruik, naamlik SST825, SST822, Krokodil, Frontana en 'n kruisingslyn met Sumai #3 agtergrond (SUM3/3/CS/LE.RA// CS/4/YANGMAI158). Drie tegnieke is gebruik naamlik watte, strooitjie en spuit-inokulasie. Die data wys duidelik dat die watte metode nog steeds die beste metode is om te gebruik om Tipe II weerstand te toets. In die tweede glashuistoets is 13 lyne van die 9de SRSN, CIMMYT, Meksiko, getoets teen die mees virulente isolaat (A191) en met een inokulasie tegniek, naamlik

watte. Die data toon aan dat daar geen betekenisvolle verskille tussen die matig vatbare SST825, lyn nr. 74 en lyn nr. 54 is nie (Tipe II weerstand). Die tweede groep kan geklassifiseer word as die matig weerstandbiedende groep wat inskrywings soos 54, 39, 21, 34, 50, 47, 19, 5, 57, 61 en 3 insluit. Nommer 14 kan geklassifiseer word as weerstandbiedend en die Sumai #3-verwante lyn as hoogs weerstandbiedend.

Twee veldproewe is uitgevoer in 2007 en 2008 onderskeidelik. Die 9de "SRSN" van CIMMYT, Meksiko, is in die veldproewe ingesluit vir beide jare. Die veldproewe se ontwerp was die "honeycomb moving grid". Die isolate wat gebruik is sluit in nommers A144, A145, A146, A149, A151, A156, A159, A161, A166, A167, A169, A176, A183, A187, A191, A194, A204 A221, A223 and A269. Die isolate was voorberei op mung boontjie-medium, geoes en are is vier keer bespuit gedurende blomstadium met 'n tussenpose van twee dae. Die omgewingstoestande vir die 2007 veldproef was uitstekend vir infeksies. Data van 2007 wys een inskrywing was vatbaar, naamlik nommer 66. Drie-en-dertig lyne het weerstand getoon, 29 lyne het matige weerstand getoon en 5 lyne het matige vatbaarheid getoon. SST806 en Marico was onderskeidelik as 'n vatbare en matig vatbare kontrole gebruik.

Addisionele inokulum is bygevoeg as gekoloniseerde graan ("grain spawn") tot die spuit inokulasie gedurende 2008. Die omgewingstoestande in 2008 was nie bevorderlik vir infeksie nie en al die inskrywings het weerstand getoon. Om hierdie rede word dit aanbeveel dat veldproewe ten minste drie jaar na mekaar uitgevoer word voordat aanbevelings gemaak kan word.