Genetic, environmental and cultural factors influencing the resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in wheat

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Genetic, environmental and cultural factors influencing the resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in wheat

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Abstract

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This thesis focuses on aspects of the resistance of wheat (*Triticum aestivum* L.) to *Mycosphaerella graminicola* (Fuckel) Schroeter (anamorph *Septoria tritici* Rob. ex Desm.), the pathogen causing leaf blotch, and the study of morphophysiological, environmental and agronomic factors which can modify its expression.

Chromosomal location of resistance was investigated in the Synthetic 6x (*Triticum dicoccoides* × *T. tauschii*) and the *Triticum aestivum* cultivar Cheyenne in the seedling stage and the adult stage, and in the *T. aestivum* cultivar Cappelle-Desprez and *Triticum spelta* in the adult stage. The Synthetic 6x showed to carry genes for resistance to the isolates IPO 92067 and IPO 93014 in the 7D chromosome in the seedling stage and the adult stage and to isolate IPO 92067 in the adult stage. Chromosomes 5A and 5D also showed to carry resistance in the adult stage to both isolates. Minor gene effects were detected on chromosome 1B of cultivar Cheyenne in the seedling stage and in chromosomes 1B and 5D in the adult stage with isolates IPO 92067 and IPO 92064. Chromosomes 2B, 3A and 3B from Cappelle-Desprez and 6D from *Triticum spelta* also showed to carry minor genes affecting resistance to both isolates in the seedling stage. Some other chromosomes showed to carry genes for resistance to only one of the two isolates.

Variation in quantitative resistance to isolate IPO 99013 was found among 50 Argentinean cultivars. Cultivars Klein Volcán, Klein Estrella and Klein Dragón showed good levels of resistance in the seedling stage and in the adult stage, whereas some other cultivars showed acceptable levels of resistance either in the seedling or in the adult stage. Significant cultivar × isolate interactions were observed in the adult stage in a data set with 16 cultivars exposed to seven isolates, although cultivars Klein Dragón and Klein Volcán showed acceptable levels of resistance to all isolates. No genetic associations between heading date, plant height and resistance to septoria tritici blotch were found in the set of 50 cultivars. Negative or positive associations between resistance and heading date were due to environmental conditions predisposing the development of the disease in early or late heading cultivars respectively in the two years experiment. Similar results were found in a set of six wheat cultivars in a two years experiment in the field. Shorter cultivars showed an increase in the severity of septoria tritici blotch under environmental conditions unconducive to the disease.

Differences in the epidemiology of the disease and their association with morphophysiological characteristics of the wheat genotypes or with weather data were also studied in near isogenic lines of the wheat cultivars Mercia and Cappelle-Desprez carrying dwarfing genes (*Rht*) or insensitivity to photoperiod (*Ppd*). Near isogenic lines of Mercia

carrying *Rht*3 or *Rht*12 genes or from Cappelle-Desprez carrying *Rht*3 gene caused the highest reduction in plant height and the highest values in the severity of septoria tritici blotch. Mercia and Cappelle-Desprez lines carrying *Ppd* genes showed a reduction in days to heading and lower severity values of septoria tritici blotch. These effects were also associated with weather conditions enhancing the development of the disease in the latest-heading cultivars.

N fertilisation caused an increase in the average severity of septoria tritici blotch when weather conditions were conducive to the development of the disease, but cultivar × N-fertilisation interactions were significant. However, the percentage of reduction in yield, yield components and test weight caused by septoria tritici blotch was similar in N-fertilised and non-fertilised conditions suggesting the presence of tolerance mechanisms. The contribution of the results described in this thesis to the existing knowledge and areas for further research are discussed.

Key words: Chromosomal location, genetic resistance, heading date, plant height, cultivar × isolate interaction, N-fertilisation, yield, yield components, septoria tritici blotch, *Mycosphaerella graminicola*, wheat.

A mis padres

Preface

This work would not have been possible without the help of many people. I especially want to thank my family and friends for their support during the periods I spent in Wageningen and during the time I was dedicated to my thesis in Argentina.

The start of this project was marked by its approval by my promotor Prof. Paul Struik from Wageningen University (WU) and my co-promotor Dr. Anthony Worland from John Innes Centre, Norwich, UK. Thank you both for your interest and determination to make things possible, for your inspiring guidance and for the critical comments on the manuscripts. Unfortunately Dr. Worland died during the preparation of this thesis. His death is an invaluable loss for the scientific community but also for his friends and colleagues as he was a special person.

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The experimental work in growth chambers, greenhouses and in the field was possible thanks to the co-operation of WU, The Netherlands, and Estación Experimental FCAyF, Los Hornos, Argentina. My special thanks go to Ing. Aad van Ast and the technical staff of the Department of Plant Sciences for their support in carrying out some of the experiments. To the former IPO-DLO (now part of Plant Research International, Wageningen) and Dr. Gert Kema and Ing. Els Verstappen for facilitating the preparation of the inocula. To Dr. C. Law (John Innes Centre, Norwich, UK) for the additional information provided about the materials. To the breeders (Buck SA, Klein SA, Thomas SA, ACA) and Ing. J. Nisi (INTA, Marcos Juárez, Argentina) for providing the seeds for the experiments. To Ing. Marcelo Asborno, Ing. Martín Pardi and the technical staff of Estación Experimental, FCAyF for their help in carrying out the field experiments.

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regression analyses.

Finally, I would like to thank all the people who shared with me my spare time in Wageningen or who supported me from Argentina, for their friendship and companionship, thus making my life far from my country much easier.

Thank you all once again,

María Rosa

Wageningen, September 2003

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CHAPTER 1

General introduction

General introduction

Bread wheat (*Triticum aestivum* L.) is the most widely grown and consumed food crop in the world. It is the staple food of nearly 35% of the world population, and the demand for wheat will grow faster than for any other major crop (Rajaram, 1999). The forecasted global demand for wheat in the year 2020 varies between 840 (Rosegrant et al., 1995) to 1050 million tonnes (Kronstad, 1998). To meet this demand, global production will need to increase 1.6 to 2.6% annually from the present production level of 560 million tonnes. For wheat, the global average yield must increase from the current 2.5 t ha^{-1} to 3.8 t ha^{-1}. In 1995, only 18 countries worldwide had an average wheat grain yield of more than 3.8 t ha^{-1}, the majority located in Northern Europe (CIMMYT, 1996). For all developing countries, wheat yields have grown at an average annual rate of over 2% between 1961 and 1994 (CIMMYT, 1996). In Western Europe and North America the annual rate of growth for yield was 2.7% from 1977 to 1985, falling to 1.5% from 1986 to 1995 (Rajaram, 1999). Argentina with a production of 14.8 million tonnes is an important wheat exporter with a volume of 8 million tonnes and an average yield of 2400 kg ha^{-1} (data from FAO 1994-2002).

Wheat breeding is focused on developing widely-adapted, disease-resistant genotypes with high yields that are stable across a wide range of environments. Incorporating durable resistance is a priority since breeding for stable yields without adequate resistance against the major diseases would be impossible (Rajaram, 1999). One of the major diseases in wheat production worldwide is septoria tritici blotch. Yield losses of 31 to 54% have been reported (Eyal et al., 1987). In Argentina, Annone et al. (1991, 1993) reported yield losses from 20 to 50% and Simón et al. (1996) found reductions in thousand kernel weight of 3 to 13%.

Background and problem definition

The disease and its dispersion

Septoria tritici blotch is caused by *Mycosphaerella graminicola* (Fuckel) Schroeter, in Cohn, which is the teleomorph stage of *Septoria tritici* Roberge ex Desmazieres (anamorph stage). Sanderson (1972) proved the connection between the two stages and the sexual (teleomorph) form has been reported in several countries (Hunter et al., 1999). The sexual stage in Argentina was reported by Cordo et al. (1990).

The sexual stage is also known to play a role in the disease cycle. It causes most of the initial infection of winter wheat crops during the autumn in the UK (Shaw and Royle, 1989), and in the USA (Schuh, 1990). In Argentina, an increase in ascospores

at harvest time has been reported, suggesting that the sexual stage may be important to initiate the infection in the next growing season (Cordo et al., 1999). Following stem elongation infection of the upper leaves of a crop has been thought to be due entirely to the asexual stage of the fungus, in which pycnidia give rise to splash-dispersed pycnidiospores, which are splash-dispersed from infected basal tissue to the upper leaves by rain drops. However, more recent work has shown that upward movement of inoculum can occur in the absence of splashy rainfall, being influenced by the position of developing leaves in relation to infected leaf layers (Lovell et al., 1997). Another possible means of spread within a crop during summer is by air-borne ascospores, which may play a more important role than previously recognised (Hunter et al., 1999).

Genetical control of resistance

Several control methods, including the use of fungicides and other cultural practices, may reduce the effect of septoria tritici blotch, but genetic resistance is the most cost-effective and environmentally safe technique to manage the disease.

Resistance conditioned by one or two genes was found in some materials (Narvaez and Caldwell, 1957; Rillo and Caldwell, 1966; Rosielle and Brown, 1979; Lee and Gough, 1984; Brading et al., 1999), whereas in some other materials at least three resistance genes have been reported (Rosielle and Brown, 1979). Most investigations have been concentrated on the analysis of complete resistance. However, quantitative resistance has been found in different genotypes (Jlibene et al., 1994; Brown et al., 2001). Most commercially grown cultivars range from moderately resistant to susceptible indicating that minor gene effects are also present. Although complete resistance is interesting because of the almost complete absence of symptoms in the host, quantitative resistance is very important because it may be more durable (Parlevliet, 1993).

Several genes for resistance to *M. graminicola* have been identified but only a few have been mapped or allocated to chromosomes. *Stb*4 in the cultivar Tadinia (Somasco et al., 1996), *Stb*5 in a Synthetic hexaploid (Arraiano et al., 2001) and *Stb*6 in the cultivar Flame (Brading et al., 2002) have been identified using single pathogen isolates. *Stb*5 and *Stb*6 have also been mapped. Quantitative trait loci (QTLs) have also been identified in some materials. Eriksen et al. (2001) found QTLs on chromosomes 2D and 3A from the resistant cultivar Senat. New sources of resistance and a deeper insight into the chromosomal location and mapping of genes and its use in marker assisted selection are essential for further progress in breeding programmes.

Complicating factors in assessing quantitative resistance

Some confounding factors in quantifying resistance are:

- The lack of relationship between plant responses in the seedling stage and in the adult stage; and
- The interaction between cultivars and isolates.

In some cultivars, resistance was found to be expressed in both the seedling stage and the adult plant stage (Somasco et al., 1996). Arama (1996) showed that there were cultivars with good resistance in both the seedling stage and the adult plant stage, other cultivars, that showed better levels of resistance in the seedling stage and again other cultivars that showed more resistance in the adult stage. Kema and Van Silfhout (1997) reported that not all isolates behaved similarly during seedling and adult plant infection. Identification of materials with resistance in both stages will be an important tool in breeding programmes.

Specific cultivar × isolate interactions have been reported by several researchers in seedlings (Eyal et al., 1985; Perelló et al., 1991; Ahmed et al., 1995; Ballantyne and Thomson, 1995; Kema et al., 1996a, b). Information on such interactions in the adult stage is scarce, although incidental reports showed that specific interactions are also present in that stage (Kema and Van Silfhout, 1997; Brown et al., 2001). However, further studies are required on cultivars with quantitative resistance to assess whether specific interactions exist.

Another complicating factor in determining resistance to septoria tritici blotch is the interaction between resistance, plant height and heading date. Several scientists reported an increased disease severity in earlier heading and shorter cultivars (Eyal et al., 1987; Van Beuningen and Kohli, 1990; Camacho Casas et al., 1995). Baltazar et al. (1990) suggested a genetic association between shortness and susceptibility, while Eyal (1981) and Rosielle and Boyd (1985) assumed a genetic association between earliness and susceptibility. Arama et al. (1999) reported no genetic association between heading date and resistance. From several investigations it is not clear if this correlation is due to genetic or epidemiological factors. More insight into these associations will allow determining whether it is possible within a given germplasm to select short, early heading, but resistant lines.

Environmental and agronomic factors modifying resistance

Expression of resistance can be modified by some cultural practices, such as N-fertilisation, which modify the microclimate within the crop canopy (Shaw and Royle, 1989) or the nitrogen concentration in the leaves (Leitch and Jenkins, 1995). However, the magnitude and direction of these effects are inconsistent. Increased N-fertility has been reported to increase the severity of the disease (Gheorghies, 1974; Prew et al.,

1983; Broscius et al., 1985; Howard et al., 1994; Leitch and Jenkins, 1995). Hayden et al. (1994) found higher severities at higher N-rates in a greenhouse study but not in the field. Johnston et al. (1979) reported a decrease in the severity of the disease with increased N in one year of their experiments. Tompkins et al. (1993) in no-till wheat and Arama (1996) found results that varied over their experiments. Most of the papers published on the influence of N on the expression of the disease are concerned with the expansion of the necrotic area or pycnidial coverage on the foliage. Few studies also include the effects of this agronomic practice on yield and yield component losses. Leitch and Jenkins (1995) observed that control of septoria tritici blotch increased yield, with the magnitude of this effect being greater at the higher rate of N applied. Johnston et al. (1979) found in crops protected by fungicide application a greater increase in yield under fertilised conditions than under non-fertilised conditions in only one out of two years. If the increase in the severity of the disease under N-fertilisation causes a reduction in yield and yield components, larger amounts of fungicides would be necessary in systems with high N-inputs.

In this context, this thesis investigates options to reduce the effects caused by the septoria tritici blotch. It is aimed to study different aspects of resistance, looking for new sources of resistance and identifying its chromosomal location. It is also aimed to investigate how genetic, cultural and environmental factors can modify the expression of this resistance.

Objectives of the research project

The objectives of the research were:

- To determine the chromosomal location of two components of the resistance to septoria tritici blotch in substitution lines of a Synthetic 6x wheat, the *Triticum aestivum* cultivars Cheyenne and Cappelle-Desprez and *Triticum spelta*.
- To evaluate variability for genetic resistance to septoria tritici blotch in Argentinean wheat cultivars in the seedling stage and the adult stage.
- To determine cultivar × isolate interactions in the adult stage in the pathosystem *Triticum aestivum/Mycosphaerella graminicola*.
- To evaluate the associations between heading date, plant height and the resistance to septoria tritici blotch in wheat cultivars and isogenic lines of wheat.
- To evaluate the effect of N-fertilisation on the susceptibility of wheat to septoria tritici blotch.
- To determine the influence of septoria tritici blotch on yield, yield components and test weight under different N-fertilisation conditions.

Outline of the thesis

In Chapter 2, research on the chromosomal location of resistance to septoria tritici blotch in the seedling stage of substitution lines of Synthetic 6x (*Triticum diccocoides* × *Triticum tauschii*; Sears, 1976), *T. spelta* and two *T. aestivum* cultivars in Chinese Spring is described. Location of resistance in the adult stage is also described for the Synthetic 6x and the *T. aestivum* cultivar Cheyenne. Data were obtained from four experiments. The first two experiments were carried out to select the sets of substitution lines and isolates of *M. graminicola* (in growth chambers in The Netherlands, in 1995 and 1999). The other two experiments were carried out to evaluate the sets of substitution lines in different environments (in growth chambers in The Netherlands in 1999 and in the field in Argentina in 2000).

Analyses of the variability in genetic resistance in the seedling and adult stages of 50 Argentinean wheat cultivars using one virulent isolate of *Mycosphaerella graminicola* in field experiments are described in Chapter 3. A set of 16 cultivars was also evaluated with seven Argentinean isolates to assess cultivar × isolate interactions. Associations between resistance, heading date and plant height were also investigated. In Chapter 3 we also address the question whether genetic or epidemiological and environmental associations exist between those traits. Experiments were carried out in The Netherlands (in greenhouses, in 1999) and in Argentina in the field in 1998 and 2000.

In Chapter 4, the influence of heading date and plant height on the resistance to septoria tritici blotch in near-isogenic lines of the wheat cultivars Cappelle-Desprez and Mercia is analysed in more detail. Field experiments were conducted in Argentina, during 2000 and 2001.

In Chapters 5 and 6, the influence of N-fertilisation on the expression of the disease is described. In Chapter 5, the progress of septoria tritici blotch under two N-fertiliser levels is analysed and in Chapter 6 the influence of septoria tritici blotch on yield, yield components and test weight under two N-fertilisation conditions is studied. Several cultivars are investigated to assess if tolerance mechanisms are present. Two field experiments were carried out in Argentina, during 1996 and 1997.

Finally, in Chapter 7, a general discussion on the main results obtained in these experiments is presented and analyses are made to what extent this thesis has produced new knowledge and insight.

CHAPTER 2

Chromosomal location of two components encoding for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in the seedling stage and in the adult stage of substitution lines of wheat[1]

[1] This chapter is based on the following publications:

M.R. Simón, A.J. Worland, C.A. Cordo, P.C. Struik. 2001. Chromosomal location of resistance to *Septoria tritici* in seedlings of a synthetic hexaploid wheat, *Triticum spelta* and two cultivars of *Triticum aestivum*. Developments in Plant Breeding. Volume 9: Wheat in a global environment. Proceedings of the 6th International Wheat Conference, 5-9 June 2000, Budapest, Hungary. Z. Bedõ, L. Lang (eds.), Kluwer Academic Publishers, Dordrecht/Boston/London, pp. 405-410.

M.R. Simón, A.J. Worland, C.A. Cordo, P.C. Struik. 2001. Chromosomal location of resistance to *Septoria tritici* in seedlings of a synthetic hexaploid wheat, *Triticum spelta* and two cultivars of *Triticum aestivum*. Euphytica 119: 149-153.

M.R. Simón, A.J. Worland, C.A. Cordo, P.C. Struik. 2001. Chromosomal location of resistance to *Septoria tritici* in adult plants of a synthetic hexaploid wheat and a wheat cultivar. XVIth Eucarpia Congress 'Plant Breeding sustaining the future', Edinburgh International Conference Centre, 10-14 September 2001, Edinburgh, Scotland, p. 30.

M.R. Simón, A.J. Worland, P.C. Struik. 2003. Chromosomal location of two components encoding for resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in seedlings and in the adult stage of substitution lines of wheat. Submitted.

Abstract

Chromosomal location of resistance expressed as reduction in necrosis percentage and pycnidial coverage in the seedling stage to two virulent Argentinean isolates of *Mycosphaerella graminicola* was studied in a synthetic hexaploid wheat (Synthetic 6x; *Triticum dicoccoides* × *Triticum tauschii*), *Triticum spelta* and the wheat (*Triticum aestivum*) cultivars Cheyenne and Cappelle-Desprez in two different environments. Substitution lines of these (resistant or moderately resistant) genotypes into (susceptible) Chinese Spring (*Triticum aestivum*) and the isolates of the fungus to be used were selected from a preliminary screening. For Synthetic 6x, resistance in the seedling stage was clearly located on chromosome 7D for both isolates. Similar results were found for *T. spelta* with one of the isolates. For Cheyenne, chromosome 1B showed to carry partial resistance, although some interactions between isolates and the environment occurred. For Cappelle, chromosomes 2B, 3A and 3B showed minor gene effects expressed as a reduction in the necrosis percentage in response to both isolates; chromosomes 1B, 2A and 5A showed similar minor gene effects in response to isolate IPO 93014. Chromosomes 5A, 2D and 6D from *T. spelta* showed to carry partial resistance to isolate IPO 92067 and 6D, 7B and 4B to isolate IPO 93014. In the adult stage, the line carrying chromosome 7D from Synthetic 6x showed a level of resistance similar to the resistant parent, whereas the lines carrying 5A or 5D showed higher resistance than Chinese Spring or even similar to Synthetic 6x to isolate IPO 92067. With isolate IPO 93014, lines carrying chromosomes 4A, 5A, 5D, 6D, 7A or 7B showed higher levels of resistance than the susceptible parent in both environments. For Cheyenne, lines carrying chromosomes 1B, 2B or 5D showed levels of resistance similar to the resistant parent or better than the susceptible parent for the average of the environments with isolate IPO 92067. Lines carrying chromosomes 1B, 5D or 6D showed higher levels of resistance than the susceptible parent or were even similar in resistance to the resistant parent with isolate IPO 92064. Significant correlations were found between both resistance components in the seedling and adult stages.

Key words: Septoria tritici blotch, *Mycosphaerella graminicola*, resistance, chromosomal location, substitution lines, *Triticum*.

Introduction

Septoria tritici blotch caused by *Mycosphaerella graminicola* (Fuckel) Schroeter in Cohn (anamorph *Septoria tritici* Rob. ex Desm.) is an important disease in many wheat-producing areas of the world and causes significant yield losses (Eyal, 1981; Eyal et al., 1987). Resistance conditioned by one or two genes was found in some materials (Narvaez and Caldwell, 1957; Rillo and Caldwell, 1966; Rosielle and Brown 1979; Lee and Cough, 1984; Brading et al., 1999), whereas in some other materials at least three resistance genes have been reported (Rosielle and Brown, 1979).

Although quantitative resistance has been found in different genotypes (Jlibene et al., 1994; Brown et al., 2001; Simón et al., 2001a) and most commercially grown cultivars range from moderately resistant to susceptible indicating that minor gene effects are also present, most investigations have concentrated on the study of major gene effects. Complete resistance is in general monogenic, race-specific and ephemeral, whereas partial resistance is generally race-non-specific, durable and oligogenic or polygenic. Although complete resistance is interesting because of the almost complete absence of symptoms in the host, partial resistance is very important due to its durability and its expression under a broad spectrum of isolates of the pathogen. A few genes may be enough to confer resistance that will hold up in farmers' fields (Dubin and Rajaram, 1996).

Several of the components of partial resistance to *M. graminicola* may be controlled by just a few genes (Jlibene and El Bouami, 1995). It would seem that those components that are genetically different could be combined into the same genetic background by crossing (Van Ginkel and Rajaram, 1999). In quantitative analyses, additive gene effects proved to contribute more to resistance than dominance effects. However, significant non-additive effects were often identified (Van Ginkel and Scharen, 1987; Bruno and Nelson, 1990; Danon and Eyal, 1990; Jonsson, 1991; Jlibene et al., 1994; Simón and Cordo, 1997, 1998). While heritabilities tend to be only moderate (Simón et al., 1998a), progress in breeding for resistance is possible.

New sources of complete and partial resistance need to be found. A few studies have been carried out to study the chromosomal location of the resistance. The increased use of molecular markers as an important tool for marker-assisted selection makes the chromosomal location more important. Once the chromosomes carrying resistance are located, finding molecular markers linked to resistance is easier through the development of recombinant lines for those specific chromosomes.

The aim of this work was:

1. To identify resistant materials in a set of accessions of *Triticum* spp. which are parents of substitution and monosomic lines.

2. To determine the chromosomal location of necrosis percentage and pycnidial coverage in the seedling stage and in the adult stage of some of the resistant materials found, using chromosome substitution lines of the susceptible *Triticum aestivum* cultivar Chinese Spring.

Materials and methods

Preliminary screening

Two preliminary screenings were carried out to select the sets of substitution lines and the isolates of the fungus to be used. The first screening included 15 parents of monosomic series or parents of substitution lines and the susceptible cultivar Shafir used as a tester. It was carried out at the former research institute IPO-DLO, The Netherlands in 1995. Genotypes were the *T. aestivum* cultivars Bezostaya, Cappelle-Desprez, Cheyenne, Chinese Spring, Favorits, Hobbit Sib, Hope, Lutescens, Mara, Poros, Shafir, Sinvalocho, Timstein, the synthetic hexaploid [(*Triticum dicoccoides* × *T. tauschii* (Sears, 1976)], *T. macha* and *T. spelta.*

The first preliminary screening was done in a growth chamber at 20-22 °C and 85-90% relative humidity in a complete randomised design with two replications in small pots. Five to ten seeds were sown per genotype per replication (pot). Plants were vernalised for one week at 4-8 °C because of the cold requirements of some genotypes. Plants were inoculated at the 1-leaf stage. Seven isolates from Argentina (IPO 86068; 92061; 92064; 92065; 92066; 92067; 93014) and three from The Netherlands (IPO 001; 290 and 323) were grown on Petri-dishes of V8 juice agar for 3 days and transferred to yeast-glucose liquid medium. Flasks were shaken for 5 days at 18 °C. Spores were suspended in distilled water and conidial suspension was adjusted to 1×10^7 spores ml^{-1}. One ml of Tween 20 per liter was added as a surfactant. After inoculation, plants were covered with transparent plastic to maintain high humidity levels. Necrosis and pycnidial coverage were scored 21-22 days after inoculations.

The second preliminary screening included 5 genotypes (Cappelle-Desprez, Cheyenne, Synthetic 6x, *T. spelta* and Chinese Spring) and 4 isolates (IPO 92064; 92065; IPO 92067 and 93014). It was planted at the Department of Plant Sciences, Wageningen University, The Netherlands, in 1999, in a growth chamber with conditions and experimental design similar to the ones in the first preliminary screening. Genotypes and isolates were selected according to their behaviour in the first preliminary screening and considering the availability of substitution lines. Plants were vernalised for three weeks at 3-4 °C. Inoculations and evaluations in the seedling stage were performed as described for the first screening experiment. At tillering,

plants were transplanted into 10-litre pots in a greenhouse at 14-17 °C and 75% relative humidity after an adaptation period of 3 days at 12 °C. Plants were inoculated at boot stage (GS 49; Zadoks et al., 1974) with the same isolates as in the seedling stage. After inoculation, plants were covered with a transparent plastic tent to maintain humidity at very high levels for 72 h. After that, conditions in the greenhouse were 18-22 °C and 80-85% humidity, humidity being maintained by means of a humidifier. Necrosis percentage and pycnidial coverage were scored 24-25 days after evaluations. Data were arcsine transformed and analysed by a combined ANOVA for both environments. A protected LSD test (P=0.05) was used for mean separation.

From these screenings, four sets of substitution lines and two isolates for each of them were chosen. Parents of these sets showed differences in resistance to septoria tritici blotch with the selected isolates at seedling stage. Furthermore, two sets were selected for evaluating resistance in the adult stage. Substitution lines were developed by C.N. Law and A.J. Worland at the John Innes Centre, Norwich, UK, and by Rosalind Morris, University of Nebraska, USA.

Final experiments

Seedling stage. Two final experiments were carried out with the 4 sets (substitutions of the 21 chromosomes of Synthetic 6x, Cheyenne, Cappelle and *T. spelta* as resistant parents in the susceptible Chinese Spring). The first experiment was planted in a growth chamber at the Department of Plant Sciences, Wageningen University, The Netherlands on 27th July 1999. The second was planted in the outdoors experimental facilities of the Facultad of Ciencias Agrarias y Forestales, La Plata, Argentina on 13th July 2000.

In both environments, the four sets of substitution lines were sown together with the parents in 10-litre pots in a randomised block design with two replications for each isolate. In each pot 6 to 8 seeds were sown. Genotypes were vernalised for 3 weeks at 4-8 °C. In 1999, the seeds were vernalised after sowing (in the growth chamber) and in 2000 in a growth chamber before sowing in pots outdoors.

The sets with Synthetic 6x, Cappelle and *T. spelta* were inoculated with the Argentinean isolates named as IPO 92067 and IPO 93014 by the former IPO-DLO, Wageningen, The Netherlands. The set with Cheyenne was inoculated with the Argentinean isolates named as IPO 92067 and IPO 92064. Isolate IPO 92064 was used instead of IPO 93014 because it gave better discrimination between the parents of the Cheyenne set.

In 1999, the isolates were grown as described for the preliminary screening experiments. In 2000, the isolates were grown on Petri-dishes of agar potato and

transferred onto malt extract agar. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in de-ionised water. The conidial suspension was adjusted in both experiments to 1×10^7 spores ml^{-1} and 1 ml of Tween 20 per liter was added as a surfactant. Plants were inoculated at the 1-leaf stage. After the inoculation, both experiments were covered with transparent plastic to maintain wet conditions for 48 h. During 1999 (growth chamber experiment), conditions after inoculation were 20-22 °C and 85-90% relative humidity. During 2000 (outdoor experiment), the average conditions after the first 48 h until evaluations were: mean temperature 12.6 °C, mean relative humidity 75% and 45 mm of rainfall distributed over 10 days.

Plants were scored 21-22 days after inoculation. Necrosis (%) and pycnidial coverage (%) were recorded. Data were arcsine transformed and analysed by a combined ANOVA for both environments. The protected LSD test (P=0.05) was used for mean separation. A correlation analysis between both resistance components was also performed.

Adult stage. Synthetic 6x/Chinese Spring and Cheyenne/Chinese Spring sets were also inoculated at the flag leaf stage (GS 49; Zadoks et al., 1974) with isolates IPO 92067 and 93014, and IPO 92067 and 92064 in 1999 (growth chamber) and 2000 (outdoors), respectively. Inoculum was prepared and conditions immediately after inoculation maintained as previously described for each environment, respectively. Conditions after inoculations in 1999 were similar to those for the seedling testing. In 2000, mean temperature after the first 48 h until evaluation was 16.9 °C, mean relative humidity 89.4% and rainfall 66.5 mm distributed over 14 days.

Data analysis in final experiments. Necrosis and pycnidial coverage were scored 25 days after inoculations on the two upper leaves of each plant. Averages of the two leaves were arcsine transformed and analysed by a combined ANOVA for both environments. The protected LSD test (P=0.05) was used for mean separation. A correlation analysis between both resistance components was also performed.

Results

Preliminary screening

The first preliminary screening (1995) in the seedling stage showed differences between genotypes, isolates and interactions between genotypes and isolates. Some materials such as the synthetic hexaploid (Synthetic 6x) proved to exhibit a very high

level of resistance – expressed as a reduction in necrosis percentage and pycnidial coverage – to the 10 isolates of *M. graminicola* tested at the seedling stage. Chinese Spring proved to be susceptible or moderately susceptible to all isolates, except to IPO 323, to which it was moderately resistant. High levels of resistance (under 20% of necrosis and under 10% pycnidial coverage) were also found in all materials, except in Hope, at least with one of the isolates (Tables 2.1 and 2.2).

Genotypes for the second preliminary screening were selected according to their behaviour in the first preliminary screening and considering the availability of substitution lines. The selected genotypes were Synthetic 6x, *Triticum spelta* and two *Triticum aestivum* cultivars (Cheyenne and Cappelle-Desprez), because they showed acceptable levels of resistance (expressed as a reduction in necrosis percentage and pycnidial coverage) with some isolates in the first preliminary screening. In addition complete sets of chromosome substitution lines from these materials into the susceptible Chinese Spring (*Triticum aestivum*) were available.

In the second preliminary screening in the seedling stage, Chinese Spring was susceptible to all isolates (IPO 92064; 92065; 92067 and 93014), Synthetic 6x, *T. spelta* and Cappelle-Desprez were resistant or moderately resistant to all isolates and Cheyenne showed variable results. In the adult stage, Synthetic 6x and Cheyenne were resistant or moderately resistant to all isolates, Chinese Spring was susceptible or moderately susceptible and Cappelle-Desprez showed variable results (Table 2.3).

Based on the results of both preliminary screenings, isolates IPO 92067 and IPO 93014 were selected for the inoculation of Synthetic 6x, Cappelle-Desprez and *T. spelta* and isolates IPO 92067 and IPO 92064 for the inoculation of Cheyenne in the seedling stage. Furthermore, isolates IPO 92067 and IPO 93014 were selected for the inoculation of Synthetic 6x and IPO 92067 and IPO 92064 for the inoculation of Cheyenne in the adult stage.

Final experiments

Seedling stage. There were significant differences for necrosis and pycnidial coverage percentages between environments (1999 and 2000) and between lines for the four sets of substitution lines with both isolates in the seedling stage. There was also a significant line × environment interaction for the set Synthetic 6x/Chinese Spring with the isolate IPO 92067 and for the set *T. spelta*/Chinese Spring with isolates IPO 92067 and IPO 93014 (Tables 2.4 and 2.5). Necrosis and pycnidial coverage percentages were higher in 1999 than in 2000. This was caused by the fact that the conditions in the 1999 growth chamber experiment were optimal for the development of the disease (Tables 2.6 and 2.7).

Table 2.1. Means of percentages of necrosis (untransformed values) caused by *Mycosphaerella graminicola* in their first screening (1995) in the seedling stage of 16 wheat genotypes with 10 isolates of the fungus (7 Argentinean and 3 Dutch).

Isolates	86068	92061	92064	92065	92066	92067	93014	001	290	323	Average genotypes
Genotypes											
Bezostaya	28.0 bcd	55.0 bc	73.0 bc	87.2 fgh	6.59 a	7.50 ab	43.7 bcde	0.62 a	59.6 c	4.35 a	36.6 c
Cappelle -Desprez	4.79 ab	5.00 ab	58.7 bc	10.0 a	21.7 abcd	15.2 abc	22.9 abcd	2.25 a	43.0 bc	61.2 cde	24.5 bc
Cheyenne	21.2 abcd	77.0 bc	29.1 ab	94.0 h	26.5 abcde	4.66 ab	71.7 ef	10.7 ab	71.2 cd	16.9 ab	42.3 de
Chinese Spring	27.2 bcd	32.5 b	71.3 bc	56.7 cdefg	45.6 bcdef	84.2 gh	60.7 def	25.6 b	69.6 cd	16.8 ab	49.0 de
Favorits	65.0 ef	72.5 bc	53.0 bc	77.5 efgh	49.0 bcdef	30.8 bcde	79.4 f	4.02 ab	70.0 cd	2.40 a	50.4 de
Hobbit Sib	0.80 a	16.2 ab	46.9 b	23.1 abc	10.4 ab	15.0 abc	15.6 ab	5.83 ab	57.5 c	68.7 de	26.0 bc
Hope	52.2 def	60.0 bc	91.7 c	64.7 cdefgh	65.0 def	77.5 fgh	77.2 ef	37.7 b	97.5 d	67.9 de	69.1 h
Lutescens	50.6 def	43.7 b	95.0 c	75.0 efgh	85.0 g	73.7 fgh	78.8 ef	15.5 ab	79.2 cd	53.3bcde	65.0 gh
Mara	3.10 ab	24.2 ab	16.5 ab	26.2 a	24.5 abcd	36.0 bcdef	26.9 abcd	0.83 a	6.90 ab	24.0 abc	18.9 bc
Poros	48.5 de	60.0 bc	91.3 c	48.0 bcdef	61.7 ef	86.0 h	60.0 c	45.2 bc	72.7 cd	3.70 a	57.7 defg
Shafir	36.9 cde	64.8 bc	82.5 bc	62.0 cdefgh	52.6 cdef	60.0 efgh	84.5 f	38.2 bc	74.5 cd	3.41 a	55.9 def
Sinvalocho	34.0 cde	44.6 b	73.3 bc	70.8 defgh	58.2 def	58.7 defgh	47.3 bcdef	15.0 ab	2.78 a	19.5 ab	42.4 cd
Synthetic 6x	6.61 ab	3.00 a	7.50 ab	3.70 a	3.62 a	0.00 a	0.50 a	0.83 a	0.00 a	0.40 a	2.61 a
Timstein	33.3 cde	68.7 bc	91.2 c	57.7 cdefgh	75.5 f	83.7 gh	72.9 ef	32.0 b	62.5 c	13.6 ab	59.1 efgh
T. macha	77.5 f	88.3 c	64.3 bc	91.7 g	43.3 abcdef	51.2 cdefgh	89.2 f	7.33 ab	63.6 cd	21.7 abc	59.8 fgh
T. spelta	10.1 ab	14.2 ab	6.91 a	12.2 ab	7.46 ab	22.0 abcde	18.5 abcde	81.6 c	77.3 cd	91.2 e	34.1 c
Average isolates	31.2 b	45.6 c	59.5 d	53.8 d	39.8 bcd	44.2 c	53.1d	20.2 a	56.7 d	29.3 ab	43.3

Means in the same column for genotypes within each isolate of the fungus and for the averages of genotypes followed by the same letter are not significantly different (LSD, P=0.05). Means in the same row for the averages of isolates of the fungus followed by the same letter are not significantly different (LSD, P=0.05).

Table 2.2. Means of percentages of pycnidial coverage (untransformed values) caused by *Mycosphaerella graminicola* in the first screening (1995) in the seedling stage of 16 wheat genotypes with 10 isolates of the fungus (7 Argentinean and 3 Dutch).

Isolates	86068	92061	92064	92065	92066	92067	93014	001	290	323	Average genotypes
Genotypes											
Bezostaya	3.75 a	45.6 cdefg	27.7 abc	72.7 g	0.98 ab	5.00 a	24.1 abcd	0.00 a	20.0 abc	0.00 a	20.0 cd
Cappelle-Desprez	0.00 a	0.20 a	11.6 ab	2.50 ab	5.13 abc	15.0 ab	30.7 bcde	0.00 a	26.5 abcd	46.2 b	13.8 bc
Cheyenne	0.00 a	61.0 g	3.34 a	63.0 eg	12.7 abcd	0.84 a	57.5 efg	2.50 abc	37.5 cd	0.50 a	23.9 de
Chinese Spring	19.3 abc	20.0 abc	36.3 bcd	50.8 defg	29.4 bcde	64.2 efg	46.4 defg	14.4 abc	43.0 cde	0.25 a	32.4 ef
Favorits	30.0 bc	35.0 bcdefg	60.6 def	67.1 fg	32.3 cde	25.0 abcd	42.2 defg	6.00 abc	36.2 cd	0.00 a	33.4 ef
Hobbit Sib	0.00 a	12.6 ab	25.0 abc	14.4 abc	4.73 abc	12.2 ab	8.32 ab	0.88 ab	30.0 bcd	52.9 c	16.1 bcd
Hope	29.1 bc	42.5 cdefg	73.0 ef	49.2 defg	42.9 ef	60.4 efg	70.2 g	28.5 bc	71.2 e	56.7 d	52.4 h
Lutescens	34.5 c	26.1 abcde	75.0 f	67.5 fg	37.2 def	67.7 fg	61.1 fg	9.0 abc	54.3 de	49.6 b	48.2 h
Mara	0.17 a	6.84 ab	2.50 a	15.6 abc	8.91abcd	16.5 abc	14.4 abc	0.00 a	5.20 ab	10.9 a	8.10 ab
Poros	34.7 c	42.0 cdefg	52.6 cdef	36.3 cde	48.3 ef	56.5 e	36.9 cdef	29.4 c	36.6 cd	0.00 a	37.3 fg
Shafir	23.7 abc	55.3 fg	66.1 ef	56.0 defg	44.3 ef	51.3 defg	67.0 g	16.2 abc	54.3 de	0.34 a	43.5 gh
Sinvalocho	13.4 abc	22.1 abcd	35.4 bcd	28.5 bcd	37.1 def	44.2 cdef	12.6 abc	3.50 abc	0.00 a	0.63 a	19.8 cd
Synthetic 6x	2.58 ab	0.00 a	3.13 a	1.25 ab	0.00 a	0.00 a	0.50 a	0.00 a	0.00 a	0.00 a	0.75 a
Timstein	20.4 abc	51.2 efg	45.0 cde	53.5 defg	63.2 f	75.0 g	57.5 efg	24.6 abc	42.5 cd	2.50 a	43.5 gh
T. macha	11.4 abc	50.0 defg	14.0 ab	16.0 abc	3.23 ab	37.5 bcde	49.7 defg	2.92 abc	14.8 abc	0.84 a	20.0 cd
T. spelta	2.29 ab	0.84 a	1.34 a	0.00 a	0.48 a	0.00 a	0.00 a	58.2 d	34.0 cd	55.7 d	15.3 bcd
Average isolates	14.1a	29.1cd	33.3 de	37.2 e	23.2 bc	33.2 de	36.2 d	12.2 a	31.6 d	17.3 ab	26.8

Means in the same column for genotypes within each isolate of the fungus and for the averages of genotypes followed by the same letter are not significantly different (LSD, P=0.05). Means in the same row for the averages of isolates of the fungus followed by the same letter are not significantly different (LSD, P=0.05).

Table 2.3. Means of percentages of necrosis (untransformed values) caused *by Mycosphaerella graminicola* in the second screening (1999) of 5 wheat genotypes with 4 Argentinean isolates of the fungus in the seedling and adult stages.

Isolates	92064		92065		92067		93014		Average genotypes	
Genotypes	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult	Seedling	Adult
Cappelle-Desprez	25.5 b	50.0 b	22.8 b	67.8 b	12.0 a	16.6 a	14.9 a	34.0 a	18.8 b	42.1 c
Cheyenne	18.6 ab	16.0 a	52.0 c	22.0 a	5.0 a	6.7 a	50.9 b	25.9 a	31.6 c	17.6 a
Chinese Spring	80.0 c	49.0 b	90.0 d	32.2 a	90.0 b	41.6 b	91.0 c	32.2 a	87.7 d	38.7 c
Synthetic 6x	4.2 a	10.0 a	3.5 a	23.7 a	1.0 a	5.0 a	3.0 a	23.7 a	2.9 a	15.6 a
T. spelta	5.4 a	35.0 b	6.0 a	32.6 a	5.0 a	8.1 a	4.0 a	45.6 a	5.1 a	30.3 b

Means followed by the same letter within the same column are not significantly different (LSD, P=0.05)

Table 2.4. Mean squares for necrosis percentage (untransformed values caused) by *Mycosphaerella graminicola* in the seedling stage of four sets of substitution lines in two environments.

Set	Synthetic 6x/Ch. Spring		Cheyenne/Ch. Spring		Cappelle-Desprez/Ch. Spring		*T. spelta*/ Ch. Spring	
Isolates	92067	93014	92067	92064	92067	93014	92067	93014
Source of variation	df							
Environment	1	14277.20 (P=0.000)[z]	4250.46 (P=0.000)	14239.4 (P=0.000)	4598.77 (P=0.000)	33622.4 (P=0.000)	6514.51 (P=0.000)	25063.70 (P=0.000)	3966.61(P=0.000)
Lines	22	946.16 (P=0.000)	972.80 (P=0.000)	594.45 (P=0.000)	652.33 (P=0.000)	508.98 (P=0.000)	570.25 (P=0.000)	629.39 (P=0.000)	435.23(P=0.000)
Environment x line	22	291.75 (P=0.010)	306.75 (P=0.327)	111.39 (P=0.145)	201.43 (P=0.265)	112.17 (P=0.225)	67.53 (P=0.492)	256.94 (P=0.000)	77.55(P=0.000)
Error	45	130.16	264.21	77.03	164.46	86.46	77.2	60.05	62.0

[z] Probability level

Table 2.5. Mean squares for pycnidial coverage percentage (untransformed values) caused by *Mycosphaerella graminicola* in the seedling stage of four sets of substitution lines intwo environments.

Set	Synthetic 6x/Ch. Spring		Cheyenne/Ch. Spring		Cappelle-Desprez/Ch. Spring		*T. spelta*/Ch. Spring	
Isolates	92067	93014	92067	92064	92067	93014	92067	93014
Source of variation	df							
Environment	1	21231.3 (P=0.000)[z]	8128.97 (P=0.000)	23342.0 (P=0.000)	3080.79 (P=0.000)	28196 (P=0.000)	6925.41 (P=0.000)	24204.60 (P=0.000)	7631.52 (P=0.000)
Lines	22	771.54 (P=0.00)	808.69 (P=0.001)	600.17 (P=0.000)	410.00 (P=0.003)	482.9 (P=0.000)	343.518 (P=0.000)	501.30 (P=0.000)	303.25 (P=0.000)
Source of variation	22	337.87 (P=0.001)	285.25 (P=0.440)	175.32 (P=0.267)	141.98 (P=0.582)	88.25 (P=0.480)	88.60 (P=0.366)	182.77 (P=0.013)	163.32 (P=0.000)
Error	45	89.75	274.13	141.83	155.82	88.02	79.30	50.01	31.17

[z] Probability level

Table 2.6. Means of percentage of necrosis (untransformed values) caused by *Mycosphaerella graminicola* in the seedling stage of Synthetic 6 x, the *T. aestivum* cultivar Cheyenne and the 21 chromosome substitution lines of these genotypes in Chinese Spring.

	Synthetic 6x/Ch. Spring						Cheyenne/Ch. Spring					
Isolates	92067			93014			92067			92064		
Lines	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	92.5 cdef	66.5 cd	79.5 bc	70.8 b	39.6 b	55.2 b	97.0 d	52.4 b	74.7 cde	99.0 d	70.0 bc	84.5 bc
1B	81.2 bcde	51.2 cd	66.2 bc	75.0 b	46.7 bc	60.8 bc	46.0 b	26.0 b	36.0 b	77.0 b	69.1 bc	73.1 b
1D	90.0 bcdef	44.7 cd	67.3 bc	89.0 bc	60.3 bcde	74.7 bcd	92.7 d	57.5 b	75.1 cde	94.1 bc	80.0 bcd	87.1 bc
2A	87.5 bcdef	70.6 cd	79.0 bc	83.3 bc	71.7 bcde	77.5 bcd	95.5 d	39.9 b	67.7 cd	100.0 d	78.7 bcd	89.4 bc
2B	88.3 bcdef	76.8 d	82.6 bc	73.0 bc	76.7 cde	74.8 bcd	88.5 cd	60.0 b	74.2 cde	97.0 cd	70.0 bc	83.5 bc
2D	98.0 ef	56.9 cd	77.4 bc	97.3 bc	85.0 de	91.2 d	96.2 d	51.5 b	73.4 cde	88.3 bc	63.0 bc	75.7 bc
3A	75.5 bc	60.0 cd	67.7 bc	93.1 bc	87.0 e	90.1 cd	80.4 c	59.7 b	70.1 cde	100.0 d	78.7 bcd	89.4 bc
3B	90.0 bcdef	38.7 abcd	64.3 bc	88.7 bc	65.0 bcde	76.9 bcd	92.5 d	61.5 c	77.0 cde	100.0 d	90.0 d	95.0 c
3D	83.7 bcde	38.3 abc	61.0 b	92.5 bc	54.2 bc	73.3 bcd	91.5 d	56.9 b	74.2 cde	94.1 bc	81.8 bcd	88.0 bc
4A	100.0 f	62.9 cd	81.4 c	95.6 bc	70.0 cde	82.8 cd	94.0 d	52.8 b	73.4 cde	100.0 d	82.0 bcd	91.0 c
4B	87.1 bcdef	50.0 cd	68.6 bc	94.0 bc	62.5 cde	78.2 bcd	77.5 cd	47.0 b	62.3 c	91.0 bc	85.0 cd	88.0 bc
4D	90.0 bcdef	39.2 abcd	64.6 bc	84.2 bc	52.5 bc	68.3 bcd	94.0 d	61.6 c	77.8 cde	100.0 d	66.2 b	83.1 bc
5A	93.7 cdef	40.0 bcd	66.8 bc	89.0 bc	75.0 cde	82.0 cd	98.3 d	60.4 c	79.4 de	97.0 cd	85.6 cd	91.3 c
5B	76.0 bcd	47.5 cd	61.7 b	99.0 c	26.2 a	62.6 bcd	95.5 d	79.6 c	87.6 e	98.0 cd	86.6 cd	91.3 c
5D	82.5 bcde	40.0 bcd	61.2 b	82.1 bc	65.0 bcde	73.6 bcd	97.5 d	45.0 b	71.0 cde	99.0 d	85.0 cd	92.0 c
6A	97.0 cdef	38.3 abc	67.6 bc	95.0 bc	50.0 bc	72.5 bcd	93.0 d	62.5 c	77.7 cde	100.0 d	93.0 d	96.5 c
6B	95.0 cdef	55.0 cd	75.0 bc	91.3 bc	66.0 cde	78.7 bcd	99.0 d	62.4 c	78.2 cde	85.1 bc	87.5 cd	86.3 bc
6D	98.7 ef	54.0 cd	76.3 bc	89.1 bc	71.0 bcde	80.1 bcd	92.5 d	47.0 b	72.2 cde	100.0 d	70.0 bc	85.0 bc
7A	66.2 b	66.7 cd	66.4 bc	85.8 bc	65.0 cde	75.4 bcd	77.5 cd	59.3 b	68.4 cd	96.2 cd	84.1 cd	90.2 c
7B	100.0 f	40.0 bcd	70.0 bc	95.1 bc	70.0 bcde	82.6 bcd	98.7 d	53.7 b	76.2 cde	100.0 d	62.5 b	81.2 bc
7D	0.78 a	10.5 a	5.64 a	0.33 a	19.4 a	9.9 a	99.0 d	45.0 b	72.0 cde	100.0 d	80.0 bcd	90.0 c
Ch. Spring	97.5 def	47.7 cd	72.6 bc	92.1 bc	83.7 de	87.9 cd	97.0 d	47.7 b	72.3 cde	96.0 cd	83.7 cd	89.9 bc
Resistant parent	1.08 a	15.0 a	8.04 a	0.95 a	27.9 a	14.4 a	5.60 a	5.20 a	5.40 a	2.00 a	26.6 a	14.3 a

Means followed by the same letter within the same column are not significantly different (LSD, P=0.05).

Table 2.7. Means of percentage of necrosis (untransformed values) caused by *Mycosphaerella graminicola* in the seedling stage of the *T. aestivum* cultivar Cappelle-Desprez, *T. spelta* and the 21 chromosome substitution lines of these genotypes in Chinese Spring.

	Cappelle-Desprez/Ch. Spring						*T. spelta*/ Ch. Spring				
Isolates	92067			93014			92067			93014	
Lines	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000
1A	84.5 bc	40.0 b	62.2 bcdef	98.0 ghij	70.0 cd	84.0 defg	80.0 def	61.6 g	70.8 g	100.0 d	83.3 de
1B	92.5 c	36.0 b	64.2 cdef	77.0 bc	35.0 b	56.0 bc	95.0 fg	40.0 defg	67.5 g	83.0 cd	77.0 de
1D	92.1 c	40.0 b	66.1 cdef	90.0 bcdefg	70.0 cd	80.0 def	100.0 g	40.0 defg	70.0 g	93.0 d	80.2 de
2A	91.0 c	42.9 b	66.9 cdef	81.0 bcd	72.5 cd	76.7 de	90.0 ef	40.0 defg	65.0 fg	85.0 cd	75.0 de
2B	85.0 bc	20.0 a	52.5 bc	76.4 bc	30.0 b	53.2 b	93.2 fg	40.0 defg	66.6 g	93.0 d	80.2 de
2D	100.0 d	45.0 b	72.5 f	95.7 fghi	80.0 d	87.9 defg	69.0 cd	16.0 abc	42.5 bc	100.0 d	80.0 de
3A	75.5 b	15.0 a	45.2 b	86.0 bcdef	59.5 c	72.7 cd	100.0 g	43.7 defg	71.8 g	91.5 cd	68.5 cd
3B	81.0 bc	30.0 ab	55.5 bc	82.9 bcde	67.5 cd	75.2 cd	100.0 g	40.0 defg	70.0 g	97.5 d	80.5 de
3D	100.0 d	40.0 b	70.0 ef	98.5 hij	80.0 d	89.2 fg	100.0 g	35.0 bcdef	67.5 g	95.0 d	80.5 de
4A	93.0 cd	31.0 ab	62.0 bcdef	93.7 efghi	72.5 cd	83.1 defg	81.5 def	47.1 fg	64.3 fg	100.0 d	80.0 de
4B	90.0 c	30.0 ab	60.0 bcdef	100.0 j	80.0 d	90.0 g	100.0 g	40.0 defg	70.0 g	74.0 c	62.0 c
4D	100.0 d	45.0 b	72.5 f	98.5 hij	80.0 d	89.3 fg	90.0 ef	36.6 bcdefg	63.3 fg	88.0 cd	72.0 cd
5A	95.5 cd	40.0 b	67.7 def	76.0 bc	72.5 cd	74.2 cd	74.0 de	30.5 abcde	52.2 cde	90.0 cd	70.0 cd
5B	99.0 cd	45.0 b	72.0 ef	90.0 bcdefg	70.0 cd	80.0 def	91.5 ef	45.3 efg	68.4 g	93.0 d	80.2 de
5D	95.1 cd	38.3 b	66.7 cdef	99.0 ij	80.0 d	89.5 fg	77.5 def	18.5 abcd	48.0 cd	97.5 d	80.5 de
6A	99.0 cd	40.0 b	69.5 ef	98.0 ghij	72.5 cd	85.2 defg	100.0 g	40.0 defg	70.0 g	97.5 d	80.5 de
6B	77.7 bc	40.0 b	58.9 bcdef	82.5 bcde	70.0 cd	76.2 de	77.5 def	47.9 fg	62.7 def	95.0 d	79.0 de
6D	94.3 cd	40.0 b	67.2 cdef	91.6 cdefgh	80.0 d	85.8 efg	45.0 bc	15.0 ab	30.0 ab	69.0 b	61.0 c
7A	88.0 c	30.0 ab	59.0 bcdef	90.5 bcdefg	67.5 cd	79.0 def	80.0 def	40.0 defg	60.0 def	91.5 cd	80.1 de
7B	94.3 cd	40.0 b	69.5 ef	93.0 defghi	70.0 cd	81.5 defg	80.0 def	38.0 cdefg	59.0 def	77.5 c	60.5 c
7D	96.0 cd	45.0 b	70.5 ef	95.0 efghi	72.5 cd	83.7 defg	35.0 b	10.0 a	22.5 a	100.0 d	90.0 e
Ch. Spring	99.0 cd	47.7 b	73.3 f	92.2 defghi	83.7 d	88.0 fg	95.0 fg	50.7 fg	72.8 g	99.0 d	79.4 de
Resistant parent	7.60 a	7.50 a	7.60 a	19.4 a	13.7 a	16.6 a	7.75 a	21.2 abcde	14.5 a	0.25 a	28.4 a

Means followed by the same letter within the same column are not significantly different (LSD, P=0.05).

For the set Synthetic 6x/Chinese Spring almost complete resistance – expressed as (almost) complete reduction in necrosis percentage (Table 2.6) and in pycnidial coverage (Table 2.8) – was located on chromosome 7D from Synthetic 6x. The substitution line carrying this chromosome showed levels of resistance similar to Synthetic 6x for both isolates in both environments and for each environment separately. For the average of both environments the line carrying chromosome 1A also showed higher resistance than Chinese Spring expressed as reduction in necrosis percentage in response to isolate IPO 93014. Some lines carrying other chromosomes showed higher levels of resistance than the susceptible parent in one environment with any of the two isolates.

For the set Cheyenne/Chinese Spring, the line carrying chromosome 1B (average of both environments) for the isolate IPO 92067 showed higher levels of resistance (expressed as reduction in necrosis percentage and pycnidial coverage) than the susceptible parent but not as high as the resistant one, suggesting the presence of partial resistance. When environments were considered separately, chromosome 1B showed higher levels of resistance than Chinese Spring expressed as reduction in the two resistant components in the growth room experiment. This line also showed a lower necrosis percentage than the susceptible parent with isolate IPO 92064 but this effect was significant only in the growth room environment. Some other chromosomes showed small effects in one of the environments (Tables 2.6 and 2.8).

For the Cappelle-Desprez/Chinese Spring set, the average of both environments showed three lines (those carrying chromosomes 2B, 3A or 3B) with higher levels of resistance than Chinese Spring, reflected in the reduction in the two resistance components for the isolate IPO 92067. For isolate 93014 also chromosomes 2B, 3A, 3B plus 1B, 2A and 5A showed higher levels of resistance – expressed as a reduction in the percentage necrosis – than the susceptible parent did for the average of both environments. Some of these effects (3A expressed as a reduction in the two resistance components, 3B expressed as a reduction in pycnidial coverage for isolate IPO 92067 and 1B and 2B expressed as a reduction in necrosis percentage for isolate IPO 93014) were more consistent because they were also present when each environment was considered separately. Although with a similar tendency as for isolate IPO 92067, no chromosomes with higher resistance than Chinese Spring (expressed as reduction in pycnidial coverage) could be detected for the average of isolate IPO 93014 over both environments, although some effects were present in any of both environments (Tables 2.7 and 2.9).

For the *T. spelta*/Chinese Spring set, the line carrying chromosome 7D showed similar levels of resistance expressed as a reduction in the two resistance components compared to the resistant parent or at least higher than the susceptible parent in both

Table 2.8. Means of percentage of pycnidial coverage (untransformed values) caused by *Mycosphaerella graminicola* in the seedling stage of Synthetic 6x, the *T. aestivum* cultivar Cheyenne and the 21 chromosome substitution lines of these genotypes in Chinese Spring.

	Synthetic 6x/Ch. Spring						Cheyenne/Ch.Spring					
Isolates	92067			93014			92067			92064		
Lines	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	88.7 bcdef	51.1 ef	69.9 bcd	46.5 b	29.3 bcd	37.9 b	97.0 ef	47.5 def	72.2 d	89.0 defg	40.0 abc	64.5 bcdefg
1B	77.5 bcde	25.0 abcde	51.2 b	69.6 bcd	40.4 cdef	55.0 bcd	37.4 b	30.0 bcde	33.7 b	63.0 bc	44.6 abcd	53.8 bcd
1D	86.5 bcdef	42.2 bcde	64.3 bcd	77.8 bcd	35.0 cde	56.4 bcd	90.2 cde	52.5 ef	71.3 cd	70.0 bcd	41.6 abc	55.8 bcd
2A	78.3 bcdef	41.5 bcde	59.9 bcd	71.3 bcd	41.3 cdef	56.3 bcd	95.5 def	55.6 f	75.6 d	88.0 efg	71.2 ef	79.6 fg
2B	82.5 bcdef	76.6 f	79.6 d	59.0bc	58.3 efg	58.7 bcd	86.0 cde	50.0 ef	68.0 cd	96.0 g	50.0 bcd	73.0 defg
2D	88.0 bcdef	33.3 bcde	60.7 bcd	84.0 cde	65.0 g	74.5 d	87.2 cde	35.7 bcdef	61.5 cd	60.0 bc	41.5 abc	50.7 bc
3A	74.6 bcd	46.2 cde	60.4 bcd	71.6 bcd	68.5 g	70.1 bcd	73.7 cd	34.8 bcdef	54.3 bcd	50.8 b	37.3 ab	44.1 ab
3B	90.0 bcdef	27.1 bcde	58.6 bcd	80.4 cde	19.5 bc	49.9 bcd	92.5 def	33.2 bcdef	62.9 cd	94.0 fg	67.9 cdef	80.9 g
3D	81.2 bcdef	21.6 abcde	51.4 b	67.5 bcd	38.3 cdef	52.9 bcd	91.5 cdef	42.9 cdef	67.2 cd	76.3 bcde	68.4 cdef	72.4 bcdefg
4A	100.0 g	34.1 bcde	67.1 cd	82.7 cde	46.2 cdef	64.5 bcd	94.0 def	28.6 bcd	61.3 cd	88.5 defg	62.0 bcde	75.2 cdefg
4B	87.1 bcdef	38.7 bcde	62.9 bcd	88.0 de	55.5 defg	71.7 cd	72.5 c	21.7 bcd	47.1 bc	66.0 bc	67.5 cdef	66.7 bcdefg
4D	87.5 bcdef	28.1bcde	57.8 bcd	59.1 bc	30.0 cd	44.6 bc	92.0 cdef	48.3 def	70.2 cd	77.0 bcde	45.0 abcd	61.0 bcdef
5A	75.0 bcd	27.5 bcde	51.2 b	78.0 bcde	38.3 cdef	58.2 bcd	88.3 cde	39.2 bcdef	63.8 cd	69.6 bcd	63.0 bcde	66.3 bcdefg
5B	71.8 bc	32.5 bcde	52.2 b	99.0 e	26.2 bc	62.6 bcd	95.5 def	48.8 def	72.2 d	91.3 efg	66.2 bcde	78.8 defg
5D	87.5 bcdef	30.0 bcde	58.7 bcd	59.5 bc	55.0 defg	57.2 bcd	91.5 cdef	31.4 bcde	61.4 cd	72.1 bcde	70.0 def	71.1 bcdefg
6A	97.0 fg	16.3 ab	56.6 bcd	87.3 cde	18.1 bc	52.7 bcd	84.0 cde	41.6 cdef	62.8 cd	74.0 bcde	85.0 f	79.5 efg
6B	89.0 bcdef	16.8 abc	52.9 b	72.5 bcd	36.5 cde	54.5 bcd	70.0 c	34.1 bcdef	52.1 bcd	69.1 bcd	66.2 bcde	67.7 bcdefg
6D	95.0 efg	37.5 bcde	66.2 bcd	59.0 bc	52.0 defg	55.5 bcd	91.2 cdef	14.4 ab	52.8 bcd	53.5 b	37.5 ab	45.5 ab
7A	63.7 b	48.0 de	55.9 bcd	73.3 bcd	34.8 cde	54.1 bcd	77.5 cd	32.7 bcdef	55.1 bcd	78.7 cdef	48.3 abcd	63.6 bcdefg
7B	92.0 defg	20.0 abcd	56.0 bcd	81.0 cde	45.0 cdef	63.0 bcd	98.7 f	38.5 bcdef	68.6 cd	85.0 cdef	48.7 abcd	66.9 bcdefg
7D	0.62 a	5.50 a	3.06 a	0.10 a	5.00 a	2.55 a	91.5 cdef	20.0 abc	55.7 cd	77.0 cdef	63.0 bcde	70.0 bcdefg
Ch. Spring	91.5 cdefg	30.0 bcde	60.7 bcd	69.0 bcd	38.5 cdef	53.7 bcd	91.5 cdef	30.0 bcde	60.7 cd	69.0 bcd	59.5 bcde	64.2 bcdefg
Resistant Parent	0.60 a	5.50 a	3.05 a	0.08 a	7.00 ab	3.54 a	0.10 a	3.5 a	1.82 a	15.0 a	21.5 a	18.2 a

Means followed by the same letter within the same column are not significantly different (LSD, P=0.05).

Table 2.9. Means of percentage of pycnidial coverage (untransformed values) caused by *Mycosphaerella graminicola* in the seedling stage of the *T. aestivum* cultivar Cappelle-Desprez, *T. spelta* and the 21 chromosome substitution lines of these genotypes in Chinese Spring.

	Cappelle-Desprez/Ch. Spring						*T.spelta*/Ch. Spring					
Isolates	92067			93014			92067			93014		
Lines	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	80.4 bcd	40.0 e	60.2 bcd	75.7 def	45.0 cd	60.4 def	70.0 d	45.0 d	57.5 fgh	91.7 h	46.2 b	68.9 i
1B	90.8 d	16.2 bcd	53.5 bcd	66.0 bcde	13.7 ab	39.9 bc	82.5 de	30.0 bcd	56.2 fgh	60.0 cde	35.0 ab	47.5 cdef
1D	86.7 bcd	35.0 de	60.8 bcd	47.5 b	40.0 c	43.7 bcde	90.0 e	30.0 bcd	60.0 h	71.7 defg	35.0 ab	53.3 defgh
2A	91.0 d	26.6 cde	58.8 bcd	72.1 cdef	62.6 d	67.4 f	80.0 de	12.5 ab	46.2 defg	84.3 gh	45.0 b	64.6 hi
2B	78.7 bcd	15.0 bc	46.9 b	68.5 bcde	15.0 a	41.7 bcde	81.6 de	13.7 ab	47.7 defg	75.0 efg	31.0 ab	53.0 defgh
2D	88.7 bcd	30.0 de	59.4 bcd	74.0 def	30.0 bc	52.0 bcdef	60.0 bcd	10.0 ab	35.0 cd	80.0 gh	35.5 ab	57.7 fgh
3A	71.0 b	15.0 bc	43.0 b	64.2 bcde	25.0 bc	44.6 bcde	82.5 de	30.0 bcd	56.2 fgh	75.0 efg	30.0 ab	52.5 cdefgh
3B	74.0 bc	20.0 bcd	47.0 b	59.7 bcde	35.0 c	47.4 bcdef	90.0 e	27.5 bcd	58.7 h	85.0 gh	34.1 ab	59.6 ghi
3D	87.0 bcd	35.0 de	61.0 cd	66.2 bcde	45.0 cd	55.6 bcdef	90.0 e	20.0 abcd	55.0 gh	55.0 bcd	35.0 ab	45.0 cdefg
4A	89.7 cd	25.0 bc	57.4 bcd	57.7 bcd	33.3 bc	45.6 bcde	71.0 d	32.5 bcd	51.7 defg	70.0 def	30.0 ab	50.0 cdefgh
4B	82.5 bcd	15.0 bc	48.7 bc	80.0 ef	45.0 cd	62.5 ef	90.0 e	20.0 abcd	55.0 gh	55.0 bcd	32.5 ab	43.7 cde
4D	89.0 cd	35.0 de	62.0 cd	85.7 f	45.0 cd	65.4 ef	73.3 de	12.5 ab	42.9 cdef	46.7 bc	32.5 ab	39.6 c
5A	86.7 bcd	35.0 de	60.8 bcd	73.0 def	45.0 cd	59.0 def	66.0 cd	16.2 abcd	41.1 cdef	71.7defg	32.5 ab	51.8 cdefg
5B	87.5 bcd	35.0 de	61.2 cd	79.5 ef	42.5 cd	61.0 ef	80.0 de	15.6 abc	47.8 defg	44.0 bcd	30.0 ab	37.0 c
5D	82.5 bcd	22.5 bc	52.5 bcd	72.0 cdef	45.0 cd	58.5 cdef	68.0 d	12.5 ab	40.2 cde	78.3 fgh	35.0 ab	56.6 fgh
6A	91.5 d	35.0 de	63.2 bcd	56.5 bcd	45.0 cd	50.7 bcdef	60.0 bcd	20.0 abcd	40.0 cde	72.0 defg	35.0 ab	53.5 defgh
6B	72.7 bc	30.0 de	51.4 bcd	79.1 ef	40.0 c	59.6 def	69.0 d	43.3 cd	56.2 fgh	61.7 cdef	35.0 ab	48.3 cdefg
6D	86.0 bcd	35.0 de	60.5 bcd	54.0 bcd	45.0 cd	49.5 bcdef	35.0 bc	10.0 ab	22.5 bc	37.5 b	15.0 a	26.2 b
7A	79.6 bcd	35.0 de	57.3 bcd	69.5 cde	45.0 cd	57.2 cdef	90.0 e	20.0 abcd	55.0 gh	75.0 efg	33.7 ab	54.4 defgh
7B	81.7 bcd	35.0 de	58.4 bcd	68.0 cde	40.0 c	54.0 bcdef	69.0 d	25.0 bcd	47.0 defg	58.0 bcde	45.0 b	51.5 cdefg
7D	84.0 bcd	28.3 cde	56.2 bcd	53.0 bc	27.5 bc	40.2 bcd	30.0 b	4.17 a	17.1 ab	82.0 gh	30.0 ab	56.0 fgh
Ch. Spring	91.5 d	42.5 e	67.0 cd	68.9 cde	42.5 cd	55.7 bcdef	78.7 de	30.0 bcd	54.4 fgh	60.0 cde	42.5 b	51.2 cdefg
Resistant parent	1.41 a	0.00 a	0.71 a	4.25 a	6.85 a	5.55 a	2.50 a	6.00 a	4.20 a	0.00 a	24.6 a	12.3 a

Means followed by the same letter in the same column are not significantly different (LSD, P=0.05).

environments and for the average of them for isolate 92067.

Lines carrying some other chromosomes such as 6D, 2D, 5A showed better resistance expressed as reduction in necrosis percentage than the susceptible parent or even similar to *T. spelta* also in the two environments and for the average of them. For isolate IPO 93014, lines carrying chromosomes 6D, 7B or 4B showed higher levels of resistance expressed as reduction in necrosis percentage than the susceptible parent did for both isolates in both environments and for the average of them. For pycnidial coverage only lines with chromosome 7D (for isolate IPO 92067) or 6D (for isolate IPO 93014) showed higher resistance than the susceptible parent for each environment and for the average of them. Some other chromosomes showed small effects in one of the environments or for the average of them (Tables 2.7 and 2.9).

There was a significant correlation between necrosis percentage and pycnidial coverage for all sets of substitution lines and for both isolates. Correlation coefficients were 0.81 for the Synthetic 6x set with isolate IPO 92067 (significant at P=0.001); 0.74 for the Synthetic 6x set with isolate IPO 93014 (significant at P=0.001); 0.88 for the Cheyenne set with isolate IPO 92067 (significant at P=0.001); 0.79 for the Cheyenne set with isolate IPO 92064 (significant at P=0.001); 0.96 for the Cappelle set with isolate IPO 92067 (significant at P=0.001); 0.81 for the Cappelle set with isolate IPO 93014 (significant at P=0.001); 0.68 for the *T. spelta* set with isolate IPO 92067 (significant at P=0.001); and 0.43 for the *T. spelta* set with isolate IPO 93014 (significant at P=0.05) (n = 46 in all cases).

Adult stage. There were significant differences for necrosis and pycnidial coverage percentages between lines for the two sets of substitution lines to both isolates. Differences between environments were also significant for the Synthetic 6x set with isolate IPO 93014 and for the Cheyenne set with both isolates (Tables 2.10 and 2.11). Necrosis and pycnidial coverage percentages were higher in 1999 than in 2000 because of the optimal conditions for the development of septoria tritici blotch in the growth chamber during 1999. For the Synthetic 6x/Chinese Spring set, several chromosomes from Synthetic 6x showed consistent resistance effects over both environments with isolate IPO 92067. The line carrying chromosome 7D of Synthetic 6x showed similar levels of resistance – expressed as reduction in necrosis and pycnidial coverage – as the resistant parent. Lines carrying chromosomes 5A or 5D also showed higher resistance than the susceptible parent or even a resistance similar to the resistant parent when the two resistance components were considered. Some other small effects were not consistent over environments. For isolate IPO 93014 lines carrying chromosomes 4A, 5A, 5D, 6D, 7A or 7B showed higher levels of resistance than Chinese Spring or even a resistance similar to Synthetic 6x (expressed as

Table 2.10. Mean squares for necrosis percentage caused by *Mycosphaerella graminicola* in the adult stage of four sets of substitution lines in two environments.

Set		Synthetic 6x/Ch. Spring		Cheyenne/Ch. Spring	
Isolates		92067	93014	92067	92064
Source of variation	df				
Lines	22	397.7 (P=0.002) [z]	532.1 (P=0.0012)	433.8 (P=0.000)	563.9 (P=0.000)
Environment	1	363.1 (P=0.125)	7439.5 (P=0.000)	2820.7 (P=0.000)	7922.1 (P=0.000)
Environment x line	22	199.1 (P=0.199)	163.2 (P=0.606)	134.3 (P=0.337)	247.6 (P=0.172)
Error	45	148.5	183.3	116.8	186.4

[z] Probability level

Table 2.11. Mean squares for pycnidial coverage percentage caused by *Mycosphaerella graminicola* in the adult stage of four sets of substitution lines in two environments.

Set		Synthetic 6x/Ch. Spring		Cheyenne/Ch. Spring	
Isolates		92067	93014	92067	92064
Source of variation	df				
Lines	22	537.9 (P=0.000) [z]	567.5 (P=0.000)	518.9 (P=0.000)	743.9 (P=0.000)
Environment	1	291.2 (P=0.195)	2385.8 (P=0.000)	4053.8 (P=0.000)	6958.0 (P=0.000)
Environment x line	22	150.8 (P=0.599)	200.3 (P=0.384)	129.1 (P=0.332)	210.9 (P=0.267)
Error	45	168.3	182.5	111.7	170.5

[z] Probability level

Table 2.12. Means of percentage of necrosis (untransformed values) caused by *Mycosphaerella graminicola* in the adult stage of Syntethic 6 x, the *T.aestivum* cultivar Cheyenne and the 21 chromosome substitution lines of these genotypes in Chinese Spring.

	Synthetic 6x/Ch. Spring						Cheyenne/Ch. Spring					
Isolates	92067			93014			92067			92064		
Lines	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	41.2 cdefg	34.6 cde	37.9 cde	78.7 fghij	63.3 h	71.0 gh	75.6 fgh	36.1 bcde	55.8 ef	47.5 bc	36.6 cdef	42.1 abcdef
1B	78.0 jk	43.1cdefg	60.6 ef	69.9 efghi	40.0 efg	54.9 cdefgh	28.7 ab	10.7 a	19.7 ab	49.0 bcd	15.0 ab	32.0 abc
1D	70.5 hijk	40.8 cdefg	55.7 def	79.2 ghij	32.5 cdef	55.8 defgh	63.7 defgh	73.7 f	68.7 f	51.7 bcd	43.3 efg	47.5 bcdefg
2A	81.8 k	31.7 cde	56.8 def	56.0 c	37.5 def	46.7 bcdefg	66.7 defghi	37.1 bcde	51.9 def	83.7 fg	18.3 abc	51.0 cdefg
2B	54.1 fgh	35.6 cde	44.8 cde	70.5 efghi	12.5 ab	41.5 bcdef	31.0 bc	26.6 bc	28.8 bc	55.0 bcd	40.0 defg	47.5 bcdefg
2D	55.0 ghi	57.2 fgh	56.1 def	88.8 j	70.0 i	79.4 h	41.5 bcd	35.6 bcde	38.6 bcde	90.7 gh	50.0 efg	70.4 gh
3A	27.5 abcde	48.7 efgh	38.1 cde	85.8 ij	17.5 abcd	51.7 bcdefg	56.0 cdef	40.0 bcde	48.0 cdef	95.8 hi	45.0 efg	70.4 gh
3B	54.9 fghi	42.0 cdefg	48.5 def	60.8 def	65.0 hi	62.9 efg	55.9 cdef	39.1 bcde	47.5 cdef	62.1 cde	61.7 g	61.9 defgh
3D	26.2 abcd	67.5 gh	46.9 cde	67.3 defgh	40.0 efg	53.6 bcdefg	71.4 efghi	40.6 bcde	56.0 ef	67.5 efg	49.2 efg	58.3 defgh
4A	30.8 bcde	43.0 cdefg	36.9 cde	51.1 b	17.5 abcd	34.3 abcde	31.2 bc	29.0 bcd	30.1 bcd	48.5 bcd	27.5 abcd	38.0 abcd
4B	76.6 jk	58.5 gh	67.6 f	65.5 defgh	40.0 efg	52.7 cdefgh	67.9 efghi	57.8 ef	62.9 f	85.0 gh	51.7 fg	68.3 fgh
4D	69.6 hij	48.3 defg	59.0 def	91.3 j	30.0 bcde	60.6 defgh	83.0 ghi	33.3 bcde	58.1 ef	58.7 cd	10.0 a	34.4 abcd
5A	27.1 abcde	30.0 bcd	28.6 bcd	25.0 b	25.0 abcde	25.0 abc	86.5 i	32.1 bcd	59.3 ef	60.0 cde	32.5 bcdef	46.2 bcdefg
5B	47.0 efg	69.6 h	58.3 def	53.0 cde	45.0 efgh	49.0 bcdefg	84.8 hi	52.5 cdef	68.6 f	92.0 gh	51.0 fg	71.5 gh
5D	13.7 a	13.7 ab	13.7 ab	38.0 bcd	10.0 a	24.0 ab	22.0 ab	33.3 bcde	27.6 bc	8.75 a	20.0 abcd	14.4 a
6A	73.7 ijk	27.1 bc	50.4 def	83.0 hi	47.5 efghi	65.2 ghi	55.4 cdef	38.4 bcde	46.9 cdef	100.0 i	45.0 efg	72.5 gh
6B	45.5 defg	58.0 gh	51.7 def	62.2 defg	60.0 ghi	61.1 defgh	54.1 cdef	20.7 ab	37.4 bcde	69.2 def	17.5 abc	43.3 abcdef
6D	34.4 bcdef	44.7 bcdef	39.6 cde	46.7 bcd	26.2 abcde	36.5 abcde	44.0 bcd	54.2 def	49.1 cdef	32.5 b	27.5 abcd	30.0 abc
7A	45.2 defg	57.0 fgh	51.1 def	56.8 cde	20.0 abcde	38.4 bcde	52.1 cde	27.5 bc	39.8 bcde	84.4 fgh	52.5 fg	68.4 fgh
7B	52.5 fg	38.4 cdef	45.5 cde	50.0 cde	15.0 abc	32.5 abcd	71.5 efghi	26.2 bc	48.8 cdef	52.5 bc	46.7 efg	49.6 bcdefg
7D	19.4 ab	12.2 ab	15.8 abc	58.0 def	35.0 def	46.5 bcdefg	60.8 defgh	50.0 cdef	55.4 def	80.0 efg	37.5 cdef	58.7 defgh
Ch. Spring	58.3 ghij	51.7 efg	55.0 def	85.2 hij	55.0 fghi	70.1 gh	58.3 def	51.7 cdef	55.0 def	80.0 efg	55.0 fg	67.5 efg
Resistant parent	22.5 a	5.00 a	13.7 a	12.7 a	7.67 ab	10.2 a	7.20 a	5.00 a	6.10 a	20.0 ab	20.0 a	20.0 ab

Means followed by the same letter are not significantly different (LSD, P=0.05)

Table 2.13. Means of percentage of pycnidial coverage (untransformed values) caused by *Mycosphaerella graminicola* in the adult stage of Synthetic 6x, the wheat cultivar Cheyenne and the 21 chromosome substitution lines of these genotypes in Chinese Spring.

	Synthetic 6x/Ch. Spring						Cheyenne/Ch. Spring					
Isolates	92067			93014			92067			92064		
Lines	1999	2000	Average	1999	2000	Average	1999	2000	Average	1999	2000	Average
1A	43.8 de	30.8 defg	37.3 cde	73.7 fgh	55.8 g	64.8 gh	61.8 efg	27.2 bcd	44.5 efgh	40.0 cd	29.1 d	34.6 bcde
1B	57.5 efg	41.2 efg	49.3 def	41.4 bcd	37.5 defgh	39.5 bcdefgh	29.7 bc	18.6 bc	24.2 b	14.8 b	10.0 bc	12.4 b
1D	70.5 fg	40.4 ef	55.4 ef	79.4 ghi	32.5 cdef	55.9 efgh	50.0 def	45.0 def	47.5 gh	47.9 cdef	38.3 defg	43.1 cdef
2A	75.5 g	28.6 defg	52.0 def	56.0 def	35.0 cdefg	45.5 cdefgh	54.8 defg	34.5 cde	44.7 efgh	72.8 ghijkl	13.3 bcd	43.1 cdef
2B	34.9 bcde	28.2 defg	31.6 cdef	29.5 bc	12.5 ab	21.0 abc	29.0 bc	29.4 bcd	29.2 bc	35.0 c	30.0 defg	32.5 bcde
2D	55.0 efg	65.0 h	60.0 ef	47.7 cde	62.5 h	55.1 efgh	38.4 bc	37.5 cdef	37.9 bcdef	90.7 l	37.5 defg	64.1 f
3A	17.5 b	46.8 fgh	32.2 cde	51.1 def	15.0 bc	33.0 bcdef	48.8 cde	31.0 bcdef	39.9 cdefg	86.8 jkl	40.0 defg	63.4 f
3B	48.3 de	31.0 defg	39.7 cde	38.3 bcd	60.0 h	49.2 cdefgh	47.3 cde	35.8 cdef	41.6 cdefg	57.1 cdefg	51.6 g	54.4 def
3D	20.2 bc	49.0 gh	34.6 cde	58.9 def	42.5 cdef	50.7 defgh	57.2 defg	31.7 bcde	44.5 efgh	67.5 hijkl	44.1 efg	558 ef
4A	35.4 cde	31.2 defg	33.3 cde	46.1 cde	15.0 bc	30.6 bcdef	30.7 bc	31.0 bcd	30.8 bcde	47.5 cde	22.5 bcd	35.0 bcde
4B	70.0 g	66.2 h	68.1 f	65.5 efg	37.5 defgh	51.5 defgh	55.7 defg	48.7 f	52.2 gh	80.0 l	44.1 efg	62.0 f
4D	53.3 ef	45.8 fgh	49.6 def	91.3 i	32.5 cdef	61.9 gh	63.9 fg	38.7 cdef	51.3 gh	57.5 defgh	7.50 b	32.5 bcd
5A	27.1 bcd	22.5 bcd	24.8 bcd	24.3 b	22.5 bcde	23.4 abcd	67.5 g	34.3 cdef	50.9 gh	60.0 defgh	27.5 cdef	43.7 cdef
5B	42.5 de	50.0 gh	46.2 def	53.0 def	37.5 defgh	45.2 cdefgh	67.1 g	42.1 def	54.6 h	85.5 kl	35.5 defg	60.5 ef
5D	2.60 a	11.2 bc	6.92 ab	13.0 ab	12.5 ab	12.7 ab	27.0 bc	32.9 bcde	30.0 bcd	8.75 b	10.0 bc	9.40 ab
6A	68.7 fg	25.1 bcde	46.9 def	82.5 hi	42.5 efgh	62.5 h	46.2 cd	37.0 cdef	41.6 cdefg	71.6 ghijkl	40.0 defg	55.8def
6B	43.8 de	28.2 cdef	36.0 cde	58.6 def	52.5 fgh	55.6 efgh	35.8 bc	26.0 bc	30.9 bcde	67.9 fghijk	15.0 bc	41.4 cdef
6D	32.3 bcd	42.2 ef	37.3 cde	18.0 ab	28.7 bcde	23.4 abcd	48.8 cde	46.0 ef	47.4 gh	15.0 b	23.7 bcd	19.4 bc
7A	40.2 d	49.0 fgh	44.6 def	52.8 def	17.5 bcd	35.2 bcdefg	45.5 cd	31.0 bcde	38.2 bcdef	74.3 ijkl	42.5 defg	58.4 ef
7B	40.8 cde	48.9 gh	44.9 def	48.3 cd	15.0 bc	31.7 bcdef	58.4 defg	28.2 bcd	43.3 cdefg	61.6 efghi	40.0 defg	50.8 def
7D	21.2 b	7.35 ab	14.3 abc	31.0 bc	32.5 cdef	31.8 bcdef	51.4 def	39.7 def	45.6 fgh	80.0 l	30.0 defg	55.0 def
Ch. Spring	60.3 ef	41.7 fg	51.0 def	48.7 cde	50.0 fgh	49.3 cdefgh	49.3 cdef	38.4 cdef	43.9 cdefg	48.7 cdef	46.3 fg	47.5 cdef
Resistant parent	4.58 a	0.00 a	2.29 a	2.58 a	10.6 a	6.60 a	6.05 a	0.00 a	3.03 a	0.10 a	0.10 a	0.10 a

Means followed by the same letter are not significantly different (LSD, P=0.05)

reduction in necrosis percentage in the two environments). Lines carrying chromosomes 5A, 5D or 6D also showed to carry resistance (expressed as reduction in pycnidial coverage). Also for this isolate some other chromosomes showed small effects in one environment only (Tables 2.12 and 2.13).

For the Cheyenne set, the line carrying chromosome 1B showed similar levels of resistance as the resistance parent with isolate IPO 92067, whereas the lines with chromosomes 2B or 5D showed higher levels of resistance than the susceptible parent (expressed as necrosis percentage for the average of the environments). When both environments were considered separately only the line with chromosome 1B showed a similar level of resistance compared to the resistant parent. Lines with some other chromosomes showed some resistance in one of the two environments. With the isolate IPO 92064, lines carrying chromosomes 1B, 5D or 6D showed similar levels of resistance as the resistant parent or a resistance higher than the susceptible parent (expressed as reduction in necrosis percentage and pycnidial coverage in both environments and for the averages of them), whereas lines with chromosomes 4A or 4D showed to carry resistance expressed as reduction in necrosis percentage. Lines with some other chromosomes only showed some levels of resistance in one of the two environments.

There was a significant correlation between necrosis percentage and pycnidial coverage for all sets of substitution lines for both isolates. Correlation coefficients were 0.95 (significant at P=0.000); 0.89 (significant at P=0.000); 0.95 (significant at P=0.000) and 0.91 (significant at P=0.000) for the Synthetic 6x (isolate IPO 92067); Synthetic 6x (isolate IPO 93014); Cheyenne (isolate IPO 92067) and Cheyenne (isolate IPO 92064) sets, respectively (n=46).

Discussion

In the preliminary screening experiments, although environmental conditions were similar for both tests, some differences in levels of resistance were observed. These might be attributed to the duration of vernalisation, which was the only factor that differed significantly among environments. In general, higher levels of resistance were found in the resistant parents in the second preliminary screening, where vernalisation was longer.

Similarly to the results found in these experiments but with different isolates, Arraiano et al. (2001) observed on detached seedling leaves, that Synthetic 6x was completely resistant to most isolates from The Netherlands and from Portugal, except IPO 92006 from Portugal whereas Chinese Spring was susceptible to all isolates

except IPO 323, to which it was moderately resistant.

Information about chromosomal location of resistance to *M. graminicola* is scarce. Resistance found in our results in lines with chromosome 7D of Synthetic 6x to both isolates was almost complete, indicating that probably only one gene confers resistance to these isolates. In line with these results, Arraiano et al. (2001), using homozygous single chromosome recombinant lines mapped a gene on the short-arm of chromosome 7D, named *Stb5*, near the centromere with the Dutch isolate IPO 94269. This may indicate that more than one gene are present in the 7D or suggests the presence of the same avirulent factors in isolates IPO 94269, IPO 92067 and IPO 93014. No information from other researchers is available about the other sets of chromosome substitution lines involved in this study.

Our results show that complete and partial resistance is present in the pathosystem *M. graminicola/T. aestivum*. Chromosome 7D from Synthetic 6x carries major genes that confer resistance to some isolates in the seedling stage, although also some minor effects were detected in one environment. In the adult stage, resistance conferred by Synthetic 6x was not so high as in the seedling stage. However, chromosomes 5A and 5D showed to carry genes providing resistance to both isolates and 7D to isolate IPO 92067. Arraiano et al. (2001) found complete resistance in the adult stage in chromosome 7D from Synthetic 6x with isolate IPO 94269.

In the seedling stage, resistance in Cheyenne and Cappelle seems to be conferred by minor genes. In the adult stage the highest levels of resistance were conferred by chromosome 1B from Cheyenne for both isolates and by 6D and 5D for one isolate, suggesting the presence of major gene effects besides some minor gene effects. *T. spelta* showed a high level of resistance to isolate IPO 92067 in chromosome 7D besides minor gene effects in some other chromosomes in the seedling stage. Some chromosomes also showed minor gene effects for isolate IPO 93014.

In spite of some differences in the chromosomes conditioning resistance expressed as necrosis percentage and as pycnidial coverage, the tendency was similar for both resistance components and they were highly correlated in the seedling stage and the adult stage. Other researchers carried out experiments under optimal environmental conditions (Eyal et al., 1987; Brown et al., 1999) and also found high correlation coefficients between both resistance components. Necrosis without pycnidia formation is mostly expressed under sub-optimal environmental conditions by resistant cultivars (Brokenshire, 1975; Eyal et al., 1987).

Location of the genes through the use of molecular markers will be the next step to incorporate this resistance in commercial materials. At present we are investigating resistance in the seedling stage in chromosome 7D of *T. spelta* and confirming the position of genes for resistance in seedling and adult stages of Synthetic 6x using

introgression lines of chromosomes 5D and 7D of Synthetic 6x in Chinese Spring. Pyramidisation of genes conditioning incomplete resistance is an important tool to get lines with durable resistance.

CHAPTER 3

Genetic variation in resistance to septoria tritici blotch (*Mycosphaerella graminicola*) and its association with genetic variation in plant height and heading date in Argentinean wheat cultivars[1]

[1] This chapter is based on the following publications:

M.R. Simón, A.E. Perelló, C.A. Cordo, S. Larrán, F.M. Ayala, D. Bayo, P.C. Struik. 2001. Associations between heading date or plant height and resistance to *Septoria tritici* blotch in the adult stage of wheat. XVI Plant Breeding Congress, Eucarpia, Edinburgh, Scotland, pp. 29-30.

M.R. Simón, A.E. Perelló, C.A. Cordo, S. Larrán, P.E.L. van der Putten, P.C. Struik. 2003. Genetic variation in resistance to septoria tritici blotch (*Mycosphaerella graminicola*) and its association with genetic variation in plant height and heading date in Argentinean wheat cultivars (submitted).

Abstract

Genetic resistance to septoria tritici blotch and its relationships with plant height and heading date were recorded in 50 wheat cultivars in three environments (two in the field and one in the greenhouse) with one virulent isolate of *Mycosphaerella graminicola* (Fuckel) Schroeter, in Cohn. Furthermore, a set of 16 cultivars was tested with 7 isolates in the greenhouse in the adult stage. Cultivars varied greatly in resistance to the disease. No genetic associations between plant height, heading date and resistance were evident. The relationships between those traits were mainly caused by environmental and epidemiological factors. Wheat cultivars with high levels of quantitative resistance to all inoculated isolates in the adult stage were identified. Specific cultivar × isolate interactions were also present.

Key words: Plant height, heading date, quantitative resistance, cultivar × isolate interactions, septoria tritici blotch, *Mycosphaerella graminicola*, *Triticum aestivum*, wheat.

Introduction

Mycosphaerella graminicola (Fuckel) Schroeter, in Cohn (anamorph *Septoria tritici*) is an important disease in many wheat-producing areas of the world and causes significant yield losses (King et al., 1983; Eyal et al., 1985, 1987; Van Ginkel and Rajaram, 1993). It is a major problem in regions characterised by a temperate, wet environment during the growing season (Eyal et al., 1987). Breeding for resistance is the most economical approach to control the disease. Resistance controlled by one major gene has been identified in some materials (Rillo and Caldwell, 1966; Rosielle and Brown, 1979; Wilson, 1979; Lee and Gough, 1984). Resistance based on several genes was also found (Rosielle and Brown, 1979). Studying the inheritance in a quantitative approach, Jlibene and El Bouami (1995) indicated that several components of the partial resistance to septoria tritici blotch may also be controlled by only one or just a few genes that could be combined into the same genetic background by crossing. Several quantitative studies have indicated the presence of general combining ability, although specific combining effects are also present (Van Ginkel and Scharen, 1987; Danon and Eyal, 1990; Jlibene et al., 1994; Simón and Cordo, 1997, 1998).

In Argentina, breeders classify most commercially grown cultivars in the range of moderately resistant to susceptible, suggesting the presence of quantitative, non-specific resistance in some of them, although isolate-specific resistance could also be present. However, an accurate characterisation is needed. Specific interaction between cultivars and isolates has been reported (Eyal et al., 1985; Perelló et al., 1991; Kema et al., 1996a, 1996b; Kema and Van Silfhout, 1997; Brown et al., 2001).

Furthermore, a complicating factor in determining resistance to septoria tritici blotch is the interaction between resistance, plant height and heading date. Several scientists reported an increased disease severity in earlier heading and shorter cultivars (Eyal et al., 1987; Van Beuningen and Kohli, 1990; Camacho Casas et al., 1995). Baltazar et al. (1990) suggested a genetic association between shortness and susceptibility, while Eyal (1981) and Rosielle and Boyd (1985) assumed a genetic association between earliness and susceptibility. Arama et al. (1999) reported no influence of heading date when cultivars were evaluated at the same developing stage under similar weather conditions. From several investigations it is not clear if this correlation is due to genetic or epidemiological factors.

The aims of this work were (i) to evaluate resistance in a broad range of wheat cultivars grown in Argentina at the seedling and adult stages to one virulent isolate of *Mycosphaerella graminicola*, (ii) to determine the relationship between resistance to *M. graminicola*, plant height and heading date in those cultivars and (iii) to evaluate a set of those cultivars at the adult stage using several Argentinean isolates.

Materials and methods

Field experiments (Experiments 1 and 2)

Fifty cultivars of wheat were selected on the basis of information provided by breeders. They differed in plant height, heading date and resistance to septoria tritici blotch, and represented a broad spectrum of those grown in Argentina in 1998. Klein Toledo, an old variety was also included, as it is known to be moderately resistant to septoria tritici blotch.

These 50 cultivars were tested in two similar field experiments (Exp. 1 in 1998 and Exp. 2 in 2000), each with three replicates. Plots consisted of three rows of 3 m long, separated by two rows of oat. Replicates were also separated by oat to avoid interplot interference. The experiments were sown on 24 June 1998 (emergence on 5 July 1998) and 5 July 2000 (emergence on 23 July 2000). At sowing, both experiments were fertilised with 50 kg ha^{-1} of P as ammonium diphosphate and 100 kg ha^{-1} N as urea.

The Argentinean isolate named IPO 99013 by the former IPO-DLO, Wageningen, The Netherlands, was grown on Petri-dishes of agar potato and transferred to malt extract agar. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in de-ionised water. The conidial suspension was adjusted for both years and both growth stages to 5×10^6 spores ml^{-1}. Tween 20 (0.5 ml per litre) was added as a surfactant. Plots were inoculated at seedling stage (2 leaves, GS 12, Zadoks et al., 1974) and at tillering stage (GS 22, Zadoks et al., 1974) in both years. After both inoculations, plants were kept moist by spraying with water several times a day for a period of 3 days. Plants were sprayed with Plantvax (oxycarboxin; 5,6 dihydro-2-methyl-N-phenyl-1, 4 oxathiin-3-carboxamide 4,4 dioxide; Dhanuka Group, New Dehli, India) when the first symptoms of leaf rust (caused by *Puccinia triticina* Eriks) appeared.

Necrosis and pycnidial coverage percentages were visually estimated in seedlings on the second leaf at 26 days after the first inoculation at the same time for all cultivars and at booting (GS 49, Zadoks et al., 1974), milk development (GS 70) and early dough stage (GS 83) at the time when each individual cultivar reached the right growth stage. For the latter three growth stages ('adult stages') evaluations were done on the three upper leaves of each plant. Twenty plants were scored in the central row of each plot at each growth stage. Plant height, measured from the soil to the flag leaf and heading date, the time from plant emergence to when 50% of the spikes emerged from the boot were also recorded in each plot. Area under disease progress curve (AUDPC) for each cultivar and each treatment was calculated to summarise the progress of the disease, according to the formula of Shaner and Finney (1977).

Weather conditions were registered at a meteorological station situated 100 m from the experiments from sowing onwards. In 1998, measurements of global radiation started on 3 September due to previous failure of the equipment.

Data were arcsine $\sqrt{x}$ transformed and analysed by ANOVA for randomised block designs. Multiple linear and non-linear regression analyses were performed with pycnidial coverage as dependent variable and heading date and plant height as independent. Data presented in the tables are the untransformed values.

Greenhouse experiments

Experiment 3. The same 50 wheat cultivars were sown in a randomised block design in a growth chamber at the Department of Plant Sciences, Wageningen University, The Netherlands. Six to eight seeds per cultivar per replication were placed in 1-l pots. Temperature was kept at 7-9 °C, relative humidity at 70-75% and photoperiod at 10 h. At tillering (GS 22, Zadoks et al., 1974), plants were transplanted to 10-l pots and transferred to the greenhouse at 15-18 °C, 70% relative humidity and more than 13 h photoperiod, after an adaptation period of three days at 12 °C. Pots were regularly watered.

At heading (GS 59, Zadoks et al., 1974) plants were inoculated with the same isolate as the one used in Exps 1 and 2 (IPO 99013), and grown as previously described. Plants were inoculated in three groups according to their heading dates and all were maintained in the same environmental conditions after inoculation. Plants were covered with a transparent plastic tent to maintain humidity at very high levels for 72 hours. After that, temperature was maintained between 17 and 22 °C and relative humidity was kept between 75 and 85%. Two humidifiers were placed in the greenhouse to maintain those high humidity levels. Although conditions were maintained as stable as possible, two cultivars Klein Volcán and Buck Ombú were sown as controls at three different dates and inoculated in the three groups to detect if any environmental variation was influencing the results.

Necrosis and pycnidial coverage percentages were evaluated on the two upper leaves 24 days after inoculations (GS 83). Data were arcsine $\sqrt{x}$ transformed and analysed by ANOVA for randomised block designs. Multiple linear and non-linear regression analyses were also done. Data presented in the tables are the untransformed values.

Experiment 4. In 1999, 16 cultivars chosen according to differences in resistance to septoria tritici blotch, heading date and plant height from Exp. 1 were sown in a factorial randomised block design with two replications in a growth chamber at the

Department of Plant Sciences, Wageningen University, The Netherlands. Factors were the 16 cultivars and 7 isolates. Six to eight seeds per treatment per replication were placed in 1-l pots. Conditions in the growth chamber and after transplanting to the greenhouse were similar to Exp. 3. Pots were regularly watered.

At heading (GS 59, Zadoks et al., 1974) plants were inoculated with 7 Argentinean isolates, named at the former IPO-DLO, Wageningen, The Netherlands, as IPO 92064; 92065; 93014; 99013; 99014; 99015 and 99016. Isolates were grown on Petri-dishes of V8 juice agar for 3 days and transferred to yeast-glucose liquid medium. Flasks were shaken for 5 days at 18 °C. Spores were resuspended in distilled water and concentration adjusted to 1×10^7 spores ml^{-1}. Tween 20 (0.5 ml per litre) was added as a surfactant. Plants were inoculated in three groups according to their heading date. The same cultivars as in the Exp. 3 were used as controls in each inoculation date and inoculated with each of the 7 isolates. After inoculation, plants were covered with a transparent plastic tent to maintain relative humidity at very high levels for 72 hours. After that, the temperature in the greenhouse was between 17 and 22 °C and the relative humidity was kept between 75 and 85% using humidifiers.

Necrosis and pycnidial coverage percentages were evaluated 24 days after inoculation (GS 83) on the two upper leaves of each plant. Data were arcsine $\sqrt{x}$ transformed and analysed by ANOVA for factorial experiments. Data presented in the tables are the untransformed values.

Results

Field experiments (Experiments 1 and 2)

Environmental conditions were more conducive to the development of the disease in 2000, mainly because of more precipitation. Periods between first inoculation to the end of evaluations were from 17 July to 22 November 1998 and from 10 August to 5 December 2000. For these periods, mean daily temperatures were 13.9 °C and 14.4 °C, mean relative humidity 85.0 and 83%, mean global radiation 4537 and 4100 W m^{-2} d^{-1} and total precipitation 198 and 388 mm for 1998 and 2000, respectively (Fig. 3.1).

In the field experiments, correlation coefficients between necrosis and pycnidial coverage percentages were high (0.81, 0.71, 0.91 and 0.85, at seedling stage, GS 49, GS 70 and GS 83, respectively, for the average of the two experiments, all of them significant at $P=0.001$; $n=50$). Due to these high correlation coefficients, only data for pycnidial coverage are given in the tables.

At all four growth stages during which observations were made, pycnidial coverage was significantly different ($P<0.001$) for cultivars and experiments, whereas

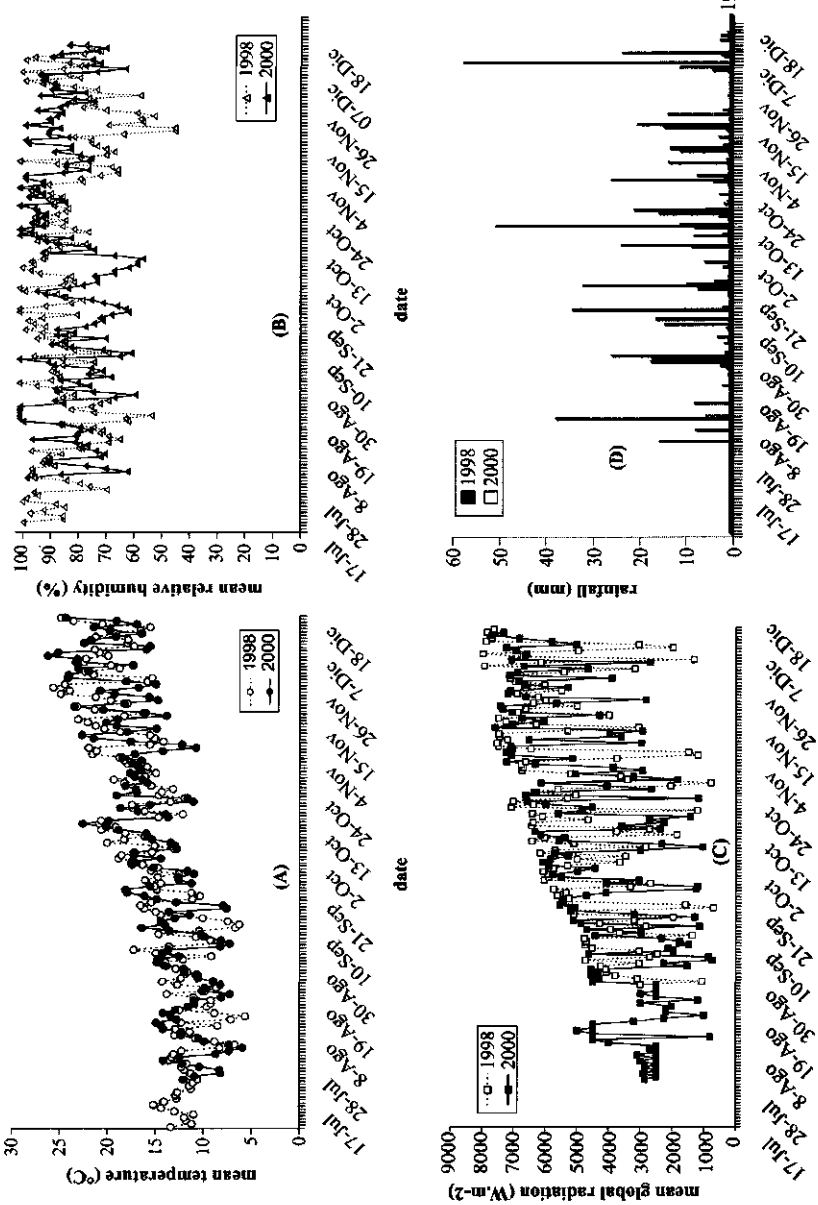

Fig. 3.1. Weather variables in the growing wheat period after inoculation with *Mycosphaerella graminicola* in two years. (A) Mean daily temperature; (B) mean relative humidity; (C) mean global radiation, (D) rainfall.

also the interaction cultivar × experiment was highly significant (P=0.000). There were also significant differences between cultivars and experiments in AUDPC, days to heading and plant height. The interaction cultivar × experiment was significant for the AUDPC (P=0.000) and days to heading (P=0.000) and almost significant (P=0.08) for plant height (Table 3.1).

In the seedling stage, pycnidial coverage percentage was slightly higher in Exp. 2 than in Exp. 1. Means of cultivars over the two experiments with isolate IPO 99013 varied from 13.6% to 73.2% of pycnidial coverage (i.e., from moderately resistant to susceptible). Cultivars Klein Estrella and Klein Volcán showed relatively good levels of resistance. In spite of some variation in cultivar behaviour between experiments, these cultivars showed acceptable levels of resistance in the two years. Some other cultivars (Klein Dragón, Buck Chambergo, ProINTA Puntal, Klein Don Enrique, Buck Panadero) also showed some level of resistance (Table 3.2).

In all adult stages during which observations were made, averages of pycnidial coverage percentage were higher in Exp. 2 than in Exp. 1. The same difference between experiments was found for the AUDPC. At GS 49 and GS 70, disease levels were very low in Exp. 1. The means for the two experiments for the average of the three upper leaves fluctuated between 0.1 and 4.3, 0.6 and 30.5, and 22.2 and 85.6% for growth stages GS 49, GS 70 and GS 83, respectively, and between 217 and 1106 for the AUDPC.

Septoria tritici blotch always reached the flag leaf at GS 83, although Klein Dragón, Klein Estrella, Klein Volcán, Cooperación Millán and Granero INTA showed very low values in that leaf (data not shown). Those cultivars also showed acceptable levels of resistance in both field experiments. Klein Toledo, the old variety known by its relatively high level of resistance to septoria tritici blotch can be considered as moderately resistance, although pycnidial coverage increased at dough stage in Exp. 2. Several other varieties which showed acceptable levels of resistance in Exp. 1 (ProINTA Quintal, ProINTA Bonaerense Redomón, Klein Granador) had an increase in the severity of septoria tritici blotch at GS 83 in Exp. 2 (Tables 3.3 and 3.4).

Pycnidial coverage percentages at the seedling and adult stages were correlated. Considering the averages of each cultivar in each experiment, the correlation was 0.34 (P=0.000; n=100). When each year was examined separately, the correlation was higher in Exp. 2 (r=0.68; P=0.000; n=50) than in Exp. 1 (r=0.26; P=0.06; n=50). Klein Estrella, Klein Volcán and Klein Dragón, which showed the best levels of resistance at the seedling stage, also showed low pycnidial coverage in the adult stage with isolate IPO 99013 and most susceptible cultivars in the seedling stage were also susceptible in the adult stage. However, some cultivars (e.g., Cooperación Millán and Granero INTA) showed good levels of resistance in the adult stage but were more susceptible in the

Table 3.1. Combined analysis of variance in 50 Argentinean wheat cultivars in two field experiments for pycnidial coverage at four different stages and for the AUDPC caused by *Mycosphaerella graminicola,* days to heading and plant height in two field experiments.

Source of variation	df	Mean squares						
		Pycnidial coverage				AUDPC	Height to flag leaf	Days to heading
		Seedling stage	GS 49[b]	GS 70	GS 83			
Cultivars	49	378.4 (P=0.000)[a]	31.1 (P=0.000)	262.9 (P=0.000)	519.0 (P=0.000)	313372 (P=0.000)	205.2 (P=0.000)	197.2 (P=0.000)
Experiment	1	646.3 (P=0.001)	1068 (P=0.000)	24003 (P=0.000)	25622 (P=0.000)	25053300(P=0.000)	2775 (P=0.000)	1236 (P=0.000)
Cultivar x Experiment	49	126.2 (P=0.000)	24.16 (P=0.000)	190.2 (P=0.000)	216.1 (P=0.000)	168245 (P=0.000)	25.6 (P=0.08)	16.0 (P=0.000)
Error	198	58.4	8.19	23.8	57.4	19173	19.0	1.79

[a] P>F

[b] GS 49; GS 70; GS 83 (Growth stages, Zadoks et al., 1974)

Table 3.2. Pycnidial coverage percentage (untransformed values) caused by *Mycosphaerella graminicola* in seedlings of 50 Argentinean wheat cultivars in two field experiments.

Cultivar	Pycnidial coverage Exp. 1 (%)	Pycnidial coverage Exp. 2 (%)	Average pycnidial coverage (%) (Exps. 1 and 2)
Bonaerense Pasuco	77.5	69.0	73.2
Bonaerense Pericón	50.7	51.8	51.2
Buck Arrayán	52.5	48.3	50.4
Buck Arriero	65.8	64.5	65.1
Buck Candil	50.9	50.1	50.5
Buck Catriel	56.8	62.7	59.7
Buck Chambergo	25.3	39.2	32.2
Buck Charrúa	39.6	54.4	47.0
Buck Fogón	29.0	42.0	35.5
Buck Guaraní	56.5	70.1	63.3
Buck Ombú	47.8	55.2	51.5
Buck Panadero	30.4	41.7	36.0
Buck Poncho	36.5	45.8	41.1
Buck Pronto	51.5	50.1	50.8
Cooperación Calquín	44.9	46.6	45.7
Cooperación Maipún	47.7	62.2	54.9
Cooperación Malambo	41.3	47.2	44.2
Cooperación Millán	64.9	43.3	54.1
Cooperación Nahuel	56.6	54.9	55.7
Granero INTA	62.0	40.3	51.1
Klein Brujo	52.8	54.1	53.4
Klein Cacique	52.5	61.7	57.1
Klein Centauro	55.4	61.4	58.4
Klein Cobre	37.5	47.4	42.4
Klein Don Enrique	49.1	21.8	35.4
Klein Dragón	36.0	19.5	27.7
Klein Estrella	2.13	25.0	13.6
Klein Granador	56.0	59.0	57.5
Klein Orión	53.7	63.3	58.5
Klein Pegaso	53.7	41.1	47.4
Klein Toledo	33.1	55.0	44.0
Klein Volcán	2.47	28.2	15.3
ProINTA Bonaerense Cauquén	59.8	57.0	58.4
ProINTA Bonaerense Redomón	61.3	63.3	62.3
ProINTA Cinco Cerros	41.0	63.1	52.0
ProINTA Elite	59.7	56.2	57.9
ProINTA Federal	54.3	34.7	44.5
ProINTA Granar	54.6	39.5	47.0
ProINTA Guazú	55.9	55.0	55.4
ProINTA Imperial	31.7	58.3	45.0
ProINTA Oasis	66.5	66.3	66.4
ProINTA Pigüe	35.5	50.6	43.0
ProINTA Puntal	18.2	47.7	32.9
ProINTA Quintal	43.9	61.2	52.6
ProINTA Real	53.7	70.8	62.2
ProINTA Super	53.8	49.3	51.6
Thomas Aconcagua	50.6	50.6	50.6
Thomas Chapelco	37.8	44.7	41.2
Thomas Nevado	53.1	78.8	65.9
Thomas Tupungato	71.9	61.8	66.8
Average experiments	47.6	51.7	49.6

LSD (P=0.05) cultivars=8.91; experiment=1.78; interaction cultivar × experiment=12.2

Table 3.3. Pycnidial coverage percentage (untransformed values) caused by *Mycosphaerella graminicola* in 50 Argentinean wheat cultivars at three adult growth stages in two field experiments.

Cultivar	GS49			GS 70			GS 83		
	Exp. 1	Exp. 2	Average (Exps. 1 and 2)	Exp. 1	Exp. 2	Average (Exps. 1 and 2)	Exp. 1	Exp. 2	Average (Exps. 1 and 2)
Bonaerense Pasuco	0.14	1.07	0.60	1.56	52.4	27.0	32.0	87.0	59.5
Bonaerense Pericón	0.28	1.47	0.87	1.23	40.4	20.8	28.8	74.7	51.7
Buck Arrayán	0.39	1.25	0.82	1.61	25.9	13.8	34.8	77.9	56.3
Buck Arriero	0.30	0.82	0.56	3.58	17.6	10.6	53.5	66.9	60.2
Buck Candil	0.96	3.04	2.00	2.09	43.8	22.9	32.5	72.8	52.6
Buck Catriel	0.40	2.13	1.26	1.54	37.1	19.3	29.9	77.3	53.6
Buck Chambergo	1.84	4.34	3.09	4.54	18.0	11.3	32.8	90.3	61.6
Buck Charrúa	1.12	1.79	1.45	7.70	20.4	14.0	30.2	72.5	51.3
Buck Fogón	0.88	4.28	2.58	2.51	25.5	14.0	44.3	72.4	58.3
Buck Guaraní	1.90	2.02	1.96	3.49	15.9	9.69	41.7	88.5	65.1
Buck Ombú	1.19	6.21	3.70	4.30	17.0	10.6	42.9	84.1	63.5
Buck Panadero	1.46	2.08	1.77	3.62	9.59	6.60	34.2	61.0	47.6
Buck Poncho	0.13	1.45	0.79	3.51	22.7	13.1	28.1	73.0	50.6
Buck Pronto	0.38	2.09	1.23	3.99	3.96	3.97	29.9	60.4	45.1
Cooperación Calquín	0.41	0.91	0.66	2.07	5.93	4.00	50.0	54.7	52.3
Cooperación Maipún	0.27	4.50	2.38	1.83	47.2	24.5	35.9	94.0	65.0
Cooperación Malambo	0.05	4.00	2.02	13.2	43.1	28.1	63.3	78.9	71.1
Cooperación Millán	0.37	0.09	0.23	1.53	10.6	6.06	29.9	22.3	26.1
Cooperación Nahuel	0.08	1.05	0.56	4.27	40.7	22.5	54.6	86.1	70.3
Granero INTA	1.50	1.52	1.51	3.14	5.09	4.11	17.5	37.0	27.2
Klein Brujo	0.49	0.50	0.49	4.62	30.9	17.8	28.9	68.3	48.6
Klein Cacique	0.36	3.65	2.00	5.85	50.1	28.0	60.5	88.5	74.5
Klein Centauro	0.06	4.21	2.13	9.02	20.1	14.6	48.7	77.2	62.9
Klein Cobre	0.53	2.56	1.54	2.42	18.7	10.6	36.7	70.7	53.7
Klein Don Enrique	0.84	0.38	0.61	7.76	2.69	5.22	52.5	41.1	46.8
Klein Dragón	0.16	0.04	0.10	0.99	3.82	2.40	16.8	27.7	22.2
Klein Estrella	0.12	0.50	0.31	0.31	4.11	2.21	22.9	25.6	24.2
Klein Granador	0.44	1.07	0.75	1.26	8.69	4.97	17.6	73.7	45.6
Klein Orión	0.87	1.72	1.29	5.33	19.5	12.4	37.9	68.8	53.3
Klein Pegaso	0.09	0.20	0.14	0.38	5.24	2.81	57.2	40.9	49.0
Klein Toledo	2.44	2.79	2.61	5.52	6.97	6.24	14.3	57.1	35.7
Klein Volcán	0.52	0.07	0.29	0.39	0.76	0.57	23.1	29.0	26.0
ProINTA Bonaerense Cauquén	0.29	6.11	3.20	2.32	23.5	12.9	32.8	87.5	60.1
ProINTA Bonaerense Redomón	0.15	2.09	1.12	1.91	24.1	13.0	26.9	71.2	49.0
ProINTA Cinco Cerros	0.19	2.63	1.41	3.79	34.0	18.9	36.9	83.0	60.0
ProINTA Elite	2.58	2.25	2.41	6.48	33.7	20.1	32.7	54.9	43.8
ProINTA Federal	0.56	0.49	0.53	4.09	5.43	4.76	65.6	64.0	64.8
ProINTA Granar	0.73	4.94	2.83	4.45	41.5	23.0	32.0	58.4	45.2
ProINTA Guazú	0.42	1.90	1.16	7.44	53.5	30.5	61.6	88.9	75.2
ProINTA Imperial	1.02	2.43	1.72	4.10	29.9	17.0	55.9	84.8	70.3
ProINTA Oasis	0.20	5.58	2.89	4.60	38.9	21.7	56.8	83.1	70.0
ProINTA Pigüe	0.47	2.33	1.40	1.91	33.5	17.7	30.9	84.7	57.8
ProINTA Puntal	0.00	0.19	0.09	1.75	13.2	7.47	52.4	67.5	59.9
ProINTA Quintal	0.26	0.34	0.30	2.29	3.20	2.74	10.5	59.6	35.1
ProINTA Real	1.06	1.02	1.04	9.54	35.3	22.4	62.6	84.2	73.4
ProINTA Super	0.24	0.84	0.54	1.40	27.4	14.4	64.9	85.4	75.1
Thomas Aconcagua	0.46	0.92	0.69	3.15	26.5	14.8	34.1	74.2	54.1
Thomas Chapelco	0.31	2.22	1.26	1.04	30.3	15.7	51.2	60.9	56.1
Thomas Nevado	0.24	5.40	2.82	2.31	47.6	25.0	78.0	93.1	85.6
Thomas Tupungato	0.08	8.60	4.34	3.81	47.8	25.8	59.5	67.2	63.3
Averages	0.60	2.28	1.44	3.63	24.5	14.1	40.2	69.0	54.6

GS 49, Zadoks et al., 1974, LSD (P=0.05) cultivar=3.24; experiment=0.65; interaction cultivar × experiment=4.58

GS 70, LSD (P=0.05) cultivar=8.58; experiment=1.71; interaction cultivar × experiment=12.1

GS 83, LSD (P=0.05) cultivar=5.52; experiment=1.10; interaction cultivar × experiment=7.81

Table 3.4. Area under disease progress curve (AUDPC) caused by *Mycosphaerella graminicola* in 50 Argentinean wheat cultivars in two field experiments.

Cultivar	AUDPC Exp. 1	AUDPC Exp. 2	Average AUDPC (Exps. 1 and 2)
Bonaerense Pasuco	282	1543	913
Bonaerense Pericón	252	1256	754
Buck Arriero	487	824	655
Buck Arrayán	307	1048	678
Buck Candil	301	1307	804
Buck Catriel	267	1229	748
Buck Chambergo	350	1046	698
Buck Charrúa	373	921	647
Buck Fogón	402	1021	711
Buck Guaraní	405	978	691
Buck Ombú	422	994	708
Buck Panadero	343	658	500
Buck Poncho	282	958	620
Buck Pronto	306	564	435
Cooperación Calquín	437	539	488
Cooperación Maipún	318	1543	931
Cooperación Malambo	718	1353	1036
Cooperación Millán	267	349	308
Cooperación Nahuel	506	1349	927
Granero INTA	202	389	296
Klein Brujo	309	1045	677
Klein Cacique	580	1540	1060
Klein Centauro	534	973	753
Klein Cobre	337	885	611
Klein Don Enrique	551	375	463
Klein Dragón	151	283	217
Klein Estrella	189	274	231
Klein Granador	165	737	451
Klein Orión	395	877	636
Klein Pegaso	465	412	438
Klein Toledo	222	591	406
Klein Volcán	195	245	220
ProINTA Bonaerense Cauquén	302	1125	713
ProINTA Bonaerense Redomón	247	971	609
ProINTA Elite	386	997	691
ProINTA Oasis	530	1332	931
ProINTA Real	662	1247	954
ProINTA Cinco Cerros	357	1228	792
ProINTA Federal	595	603	599
ProINTA Granar	333	1171	752
ProINTA Guazú	615	1583	1099
ProINTA Imperial	521	1176	848
ProINTA Pigüe	281	1232	756
ProINTA Puntal	447	753	600
ProINTA Quintal	123	531	327
ProINTA Super	544	1129	836
Thomas Aconcagua	326	1024	675
Thomas Chapelco	428	990	709
Thomas Nevado	663	1549	1106
Thomas Tupungato	537	1372	954
Averages	384	962	673

LSD (P=0.05) cultivar=157; experiment=31.3; interaction cultivar × experiment=222

Table 3.5. Days to heading and plant height of 50 Argentinean wheat cultivars in two field experiments.

Cultivar	Days to heading (cm)			Plant height (cm)		
	Exp. 1	Exp. 2	Average Exps.	Exp. 1	Exp. 2	Average Exps.
Bonaerense Pasuco	103	103	103	73	70	71
Bonaerense Pericón	102	104	103	75	71	73
Buck Arrayán	106	102	104	67	67	67
Buck Arriero	108	103	105	69	67	68
Buck Candil	110	105	107	63	61	62
Buck Catriel	112	106	109	78	73	75
Buck Chambergo	96	92	94	65	56	60
Buck Charrúa	111	103	107	75	75	75
Buck Fogón	104	101	102	71	69	70
Buck Guaraní	94	91	92	67	60	63
Buck Ombú	92	90	91	66	55	60
Buck Panadero	103	100	101	71	71	71
Buck Poncho	102	100	101	70	66	68
Buck Pronto	91	90	90	70	61	65
Cooperación Calquín	96	90	93	66	59	62
Cooperación Maipún	106	99	102	62	56	59
Cooperación Malambo	101	101	101	63	60	61
Cooperación Millán	99	94	96	66	65	65
Cooperación Nahuel	105	102	103	71	67	69
Granero INTA	91	91	91	64	60	62
Klein Brujo	98	94	96	74	63	68
Klein Cacique	106	101	103	87	82	84
Klein Centauro	103	100	101	80	76	78
Klein Cobre	93	89	91	66	62	64
Klein Don Enrique	97	90	93	67	54	60
Klein Dragón	97	92	94	84	74	79
Klein Estrella	109	104	106	71	59	65
Klein Granador	97	91	94	92	73	82
Klein Orión	93	90	91	74	65	69
Klein Pegaso	106	101	103	74	70	72
Klein Toledo	89	89	89	82	70	76
Klein Volcán	101	94	97	81	65	73
ProINTA Bonaerense Cauquén	105	101	103	73	68	70
ProINTA Bonaerense Redomón	109	105	107	76	72	74
ProINTA Cinco Cerros	107	105	106	77	68	72
ProINTA Elite	95	93	94	68	62	65
ProINTA Federal	96	89	92	65	59	62
ProINTA Granar	96	93	94	68	63	65
ProINTA Guazú	101	102	101	69	68	68
ProINTA Imperial	95	90	92	74	68	71
ProINTA Oasis	102	96	99	67	60	63
ProINTA Pigüe	105	101	103	77	68	72
ProINTA Puntal	111	95	103	66	65	65
ProINTA Quintal	94	90	92	75	67	71
ProINTA Real	96	94	95	61	59	60
ProINTA Super	111	97	104	74	63	68
Thomas Aconcagua	106	103	104	71	69	70
Thomas Chapelco	111	104	107	78	71	74
Thomas Nevado	100	98	99	73	64	68
Thomas Tupungato	103	102	102	68	65	66
Average	101	97	99	72	66	69

Days to heading: LSD (P=0.05) cultivar=1.5; experiment=0.3; interaction cultivar × experiment=2.1.
Height to flag leaf: LSD (P=0.05) cultivar =4.9; experiment=1.0.;
interaction cultivar × experiment=7.0

seedling stage. Some other cultivars (e.g., Buck Chambergo) with acceptable levels of resistance in seedlings were more susceptible in the adult stage, especially in Exp. 2.

For the average of both experiments differences in heading date and plant height between the extreme cultivars were 20 days (from 89 to 109 days) and 25 cm (from 59 to 84 cm), respectively. Cultivars with relatively high levels of resistance to isolate IPO 99013 were found among those with early heading date and short stature. Klein Estrella is a late cultivar (heading date 106 days averaged over the two experiments), with intermediate plant height (65 cm to the flag leaf averaged over the two experiments). Klein Volcán and Klein Dragón are early heading (97 and 94 days, resp.) with intermediate to high plant height (73 and 79 cm, resp.). Cooperación Millán and Granero INTA which showed acceptable levels of resistance in the adult stage are early heading cultivars (96 and 91 days, resp.) with intermediate to low plant height (65 and 62 cm, resp.). Klein Toledo, the earliest cultivar in the field experiments has been considered for many years as one of the most resistant varieties in Argentina and showed acceptable levels of resistance in the adult stage in these experiments (Table 3.5).

Multiple linear regression analysis between pycnidial coverage as dependent variable and plant height and heading date as independent variables, yielded significant R^2 values in Exp. 1 for the growth stages GS 49, GS 70 and GS 83 (Table 3.6), but not for the seedling stage. Also the multiple linear regression with the AUDPC as the dependent variable was significant in Exp. 1. If the R^2 values and the regression coefficients were statistically significantly different from 0, the regression coefficients were always negative, for the days to heading and for the plant height. In Exp. 2, however, the R^2 values were only significant for the pycnidial coverage at GS 70 and for the AUDPC. In those two cases the regression coefficients were positive and significant for days to heading, but statistically not significant for plant height. We also tested a large set of multiple non-linear regression models, with different numbers of predictors and different powers of these predictors. However, only for GS 83 and for the AUDPC in Exp. 1 we were able to identify models with slightly higher probabilities of the R^2 values than for the multiple linear models. These models were:

necrosis% (GS 83) = 269 (P=0.017) $- 8.19x_2$ (P = 0.018) +
$0.0666x_1x_2$ (P=0.018) $- 0.0001430x_1^3$ (P=0.030) +
$0.00006x_2^3$ (P=0.521); R^2=23.90 (P=0.014)

and

necrosis% (AUDPC) = 4159 (P=0.015) $- 126.4x_2$ (P=0.016) +
$0.976x_1x_2$ (P=0.022) $- 0.002183x_1^3$ (P=0.028) +
$0.00128x_2^3$ (P=0.393); R^2=21.37 (P=0.026)

with x_1=days to heading and x_2=plant height. These equations indicate that plant height and heading date were negatively associated and that the multiplicative coefficient (x_1x_2) was positively associated (due to the negative effects of plant height and heading date).

Greenhouse experiments

Experiment 3. Controls inoculated in the three groups differing in heading date showed similar values of necrosis and pycnidial coverage. For that reason, values were not adjusted with the controls. There was a high correlation between both disease parameters, mainly attributed to the appropriate conditions (temperature and humidity) for the development of septoria tritici blotch.

There were significant differences between cultivars for both resistance components. Necrosis percentage fluctuated between 17.6% and 76.3% and pycnidial coverage between 15.6 and 69.7% for all cultivars. The most resistant cultivars in the field also showed low disease values in Exp. 3. Klein Dragón, Klein Volcán, Klein Estrella and Granero INTA showed the best resistance levels (Table 3.7). Multiple

Table 3.6. Multiple linear regression on pycnidial coverage caused by *Mycosphaerella graminicola* as dependent variable and with days to heading and plant height as independent variables for 50 Argentinean wheat cultivars at seedling stage, boot stage (GS 49), milk development (GS 70) and early dough stage (GS 83), and for the area under disease progress curve (AUDPC) in Exps 1 and 2.

Growth stage /AUDPC	Constant	Days to heading	Plant height	R^2
Experiment 1				
Seedling	62.7 (P=0.020)	−0.06 (P=0.820)	−0.19 (P=0.370)	2.03 (P=0.620)
GS 49	24.8 (P=0.000)	−0.19 (P=0.000)	−0.03 (P=0.370)	33.4 (P=0.000)
GS 70	37.9 (P=0.000)	−0.18 (P=0.040)	−0.14 (P=0.090)	15.4 (P=0.020)
GS 83	35.3 (P=0.170)	0.38 (P=0.080)	−0.48 (P=0.020)	14.2 (P=0.030)
AUDPC	636 (P=0.090)	2.68 (P=0.410)	−7.30 (P=0.020)	11.2 (P=0.060)
Experiment 2				
Seedling	14.6 (P=0.450)	0.22 (P=0.310)	0.16 (P=0.450)	5.90 (P=0.240)
GS 49	−0.55 (P=0.950)	0.15 (P=0.170)	−0.10 (P=0.340)	4.17 (P=0.370)
GS 70	−76.6 (P=0.004)	1.24 (P=0.000)	−0.25 (P=0.350)	30.9 (P=0.000)
GS 83	4.04 (P=0.910)	0.73 (P=0.090)	−0.09 (P=0.830)	6.7 (P=0.200)
AUDPC	−2031 (P=0.030)	31.1 (P=0.0014)	0.04 (P=1.000)	22.0 (P=0.003)

Table 3.7. Necrosis and pycnidial coverage percentage (untransformed values) caused by *Mycosphaerella graminicola* in 50 Argentinean wheat cultivars with isolate IPO 99013 in the greenhouse (Exp. 3), days to heading and plant height to flag leaf (cm).

Cultivar	Necrosis (%)	Pycnidial coverage (%)	Days to heading	Height to flag leaf
Bonaerense Pasuco	50.2	41.8	92.0	64.0
Bonaerense Pericón	40.2	33.4	90.0	66.0
Buck Arriero	44.7	34.4	95.5	60.0
Buck Arrayán	55.6	40.4	94.0	56.0
Buck Candil	40.1	34.3	98.5	51.5
Buck Catriel	35.9	31.4	100	66.5
Buck Chambergo	40.1	32.7	85.5	54.5
Buck Charrúa	41.0	32.8	99.0	64.0
Buck Fogón	55.7	42.9	92.0	62.0
Buck Guaraní	52.5	42.9	84.5	58.5
Buck Ombú	52.8	43.0	84.5	58.0
Buck Panadero	35.0	32.9	91.0	59.5
Buck Poncho	35.1	30.9	90.0	59.0
Buck Pronto	35.6	32.8	83.0	59.5
Cooperación Calquín	50.1	42.7	84.5	56.5
Cooperación Maipún	56.3	50.5	94.5	54.0
Cooperación Malambo	70.0	60.6	89.0	53.5
Cooperación Millán	32.4	30.1	88.5	56.0
Cooperación Nahuel	60.1	52.9	94.0	61.0
Granero INTA	27.0	25.2	83.0	53.0
Klein Brujo	32.2	30.0	90.5	63.0
Klein Cacique	60.4	57.2	94.0	77.5
Klein Centauro	50.2	44.2	91.0	70.5
Klein Cobre	45.8	37.7	84.5	54.5
Klein Don Enrique	50.1	41.7	86.5	58.5
Klein Dragón	17.6	15.6	87.0	74.5
Klein Estrella	26.0	22.7	97.5	60.0
Klein Granador	35.6	31.6	86.5	76.0
Klein Orión	47.5	44.1	85.5	66.5
Klein Pegaso	50.3	42.7	94.0	65.5
Klein Toledo	30.2	26.4	80.5	70.5
Klein Volcán	25.1	20.5	89.5	72.0
ProINTA Bonaerense Cauquén	45.6	35.7	93.0	62.0
ProINTA Bonaerense Redomón	45.6	36.6	97.0	65.5
ProINTA Cinco Cerros	50.1	40.3	85.0	58.0
ProINTA Elite	40.1	35.6	90.5	57.5
ProINTA Federal	40.1	35.2	86.0	50.0
ProINTA Granar	35.0	33.1	95.0	65.5
ProINTA Guazú	70.0	61.8	86.0	55.0
ProINTA Imperial	60.2	53.4	86.0	59.0
ProINTA Oasis	68.4	58.9	89.0	60.5
ProINTA Pigüe	45.6	40.9	84.5	63.5
ProINTA Puntal	46.6	39.5	93.0	68.0
ProINTA Quintal	30.1	27.4	99.5	56.0
ProINTA Real	72.5	64.0	84.0	65.5
ProINTA Super	70.1	63.3	99.0	65.0
Thomas Aconcagua	45.6	41.7	94.5	60.0
Thomas Chapelco	56.4	52.4	99.5	69.0
Thomas Nevado	76.3	69.7	90.0	64.0
Thomas Tupungato	60.2	53.4	91.0	59.5

LSD (P=0.05) cultivar for necrosis percentage=14.70; for pycnidial coverage= 0.98.

linear regression analysis showed no association between heading date and plant height with necrosis or pycnidial coverage percentage.

Multiple linear equation for pycnidial coverage was:

pycnidial coverage (%) = 40.97 (P=0.05) + 0.04 x_1 (P=0.86) − 0.08 x_2 (P=0.65); R^2= 0.47% (P=0.89)

The most significant multiple non-linear regression equation was

pycnidial coverage (%) = 85.9 (P=0.272) − 3.56x_2 (P=0.228) + 0.0535x_1x_2 (P=0.151) − 0.000133x_1^3 (P=0.201) − 0.000109x_2^3 (P=0.331); R^2 = 9.91% (P=0.310)

with x_1=days to heading and x_2=plant height.

Experiment 4. Percentages of necrosis and pycnidial coverage are also presented for Exp. 4. Significant differences were found for both disease parameters between cultivars and isolates, whereas also the interaction cultivar × isolate was highly significant (Table 3.8). For necrosis percentage, Klein Dragón and Klein Volcán and then ProINTA Quintal and Buck Poncho showed the best levels of resistance for the average of the seven isolates. For Klein Dragón necrosis percentage varied between 7.5 and 35.1% for all isolates and for Klein Volcán between 7.0 and 26.8%. For pycnidial coverage Klein Dragón, ProINTA Quintal and Klein Volcán showed the best values for the average of the isolates. Considering each particular isolate Klein Dragón varied between 0.5 and 32.6%; Klein Volcán between 0.9 and 23.7% and ProINTA Quintal between 0.2 and 28.4% (Table 3.9).

For the whole set of cultivars and isolates, the correlation between necrosis and pycnidial coverage was 0.86 (n=112, significant at P=0.001). Some cases, such as Buck Panadero with isolates IPO 92064 and 92065, showed lower values for pycnidial coverage than expected based on the values for the percentage of necrosis. Moreover,

Table 3.8. Analysis of variance for necrosis and pycnidial coverage percentage (untransformed values) caused by *Mycosphaerella graminicola* in 16 wheat cultivars with 7 isolates (Exp. 4).

Source of variation	df	Mean squares (P>F)			
		Necrosis percentage		Pycnidial coverage	
Cultivars	15	1307	(P=0.000)	935.8	(P=0.000)
Isolates	6	1213	(P=0.000)	2150	(P=0.000)
Cultivar × isolate	90	251.4	(P=0.000)	193.1	(P=0.000)
Error	111	24.0		36.8	

some cases showed slightly higher pycnidial coverage values than expected based on necrosis percentage (Klein Estrella with isolate IPO 99015 and Buck Guaraní with isolate IPO 99016).

Discussion

Pycnidial coverage percentages in seedlings and in adult plants were correlated in the field using the isolate IPO 99013. However, there were cultivars with combined seedling and adult plant resistance, whereas others showed better levels of resistance either in the seedling or in the adult stage. Arama (1996) found similar results. Kema and Van Silfhout (1997) observed that in general adult plants were more susceptible than seedlings, although not all isolates responded similarly to seedling and adult plant infection.

Pycnidial coverage was highly correlated with necrosis percentage in both field and greenhouse experiments. This is in agreement with previous findings for field experiments (Arama, 1996; Brown et al., 2001), although in some cultivar × isolate combinations high percentages of necrosis with low pycnidial coverage have been found. In the present work, there were also some cultivars that showed higher pycnidial coverage than necrosis due to the presence of pycnidia in green areas. In greenhouses, sometimes the correlation between the two disease parameters was not high (Arama, 1996). In our greenhouse experiment relative humidity was kept as high as possible during the period after inoculation by means of humidifiers. Although pycnidial coverage is considered more accurate because senescence and other diseases do not interfere in the results, our and previous investigations indicate that especially in field conditions, necrosis percentage can also be a good predictor of resistance, and is easier to be measured (Brown et al., 2001).

Variation in genetic resistance measured as percentage of pycnidial coverage within a wide spectrum of cultivars grown in Argentina during 1998 and the old cultivar Klein Toledo was found. Cultivars can be classified from moderately resistant to susceptible with the Argentinean isolate IPO 99013 based on three experimental environments (two assays in the field and one in the greenhouse).

When a set of 16 cultivars was tested with 7 isolates in the adult stage, two of them (Klein Volcán and Klein Dragón) showed good levels of resistance with all isolates. Although more isolates should be used to allow for such statements, our finding suggests that non-specific resistance may be present in those cultivars in the adult stage. In the seedling stage, these cultivars also showed a high level of resistance with IPO 99103. Specific interactions have been reported by several researchers in

Table 3.9. Means of necrosis (N) and pycnidial coverage percentage (P) (untransformed values) caused by *Mycosphaerella gramicola* of 16 Argentinean wheat cultivars with 7 Argentinean isolates in the greenhouse (Exp. 4).

Isolate	92064		92065		93014		99013		99014		99015		99016		Average	
Cultivar	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)	N (%)	P (%)
Buck Arriero	18.0	16.8	33.3	13.3	21.8	3.90	44.7	34.4	34.5	30.4	14.6	8.05	30.8	14.2	28.2	17.3
Buck Charrúa	51.3	20.2	45.7	28.7	22.6	14.8	41.0	32.8	33.0	32.4	10.3	5.80	33.2	16.3	33.9	21.5
Buck Guaraní	11.8	2.73	41.0	30.0	17.6	18.6	52.5	42.9	49.9	43.6	6.80	4.45	21.6	23.2	28.7	23.6
Buck Ombú	83.7	61.6	28.3	12.2	36.7	11.4	52.8	43.0	89.1	70.0	46.7	23.0	91.4	59.4	61.2	40.1
Buck Panadero	30.9	4.20	50.6	13.7	21.6	18.1	35.0	32.9	88.9	70.4	27.7	26.7	57.6	34.7	44.6	28.7
Buck Poncho	15.2	12.2	14.1	13.7	25.8	22.6	35.1	30.9	34.7	29.1	17.6	13.3	41.0	55.7	26.2	25.4
Klein Dragón	11.1	4.10	7.70	1.00	7.50	7.10	17.6	15.6	35.1	32.6	8.75	0.50	8.70	4.00	13.8	9.30
Klein Estrella	31.1	20.0	60.7	15.0	35.0	25.0	26.0	22.7	35.0	27.5	56.1	60.0	30.0	22.5	39.1	27.5
Klein Orión	45.6	25.0	24.0	17.2	16.8	5.40	47.5	44.1	86.6	67.1	45.0	30.0	35.0	30.0	43.1	31.3
Klein Volcán	12.1	11.5	7.00	0.90	26.8	22.0	25.1	20.5	24.1	23.7	10.2	6.80	22.2	11.2	18.2	13.8
ProINTA Oasis	44.1	18.7	21.8	6.34	9.30	4.40	68.4	58.9	64.4	64.4	38.8	26.7	10.2	3.90	36.7	26.2
ProINTA Puntal	37.5	25.0	50.0	25.0	68.5	50.0	46.6	39.5	42.5	32.5	50.0	40.0	37.5	32.5	47.5	34.9
ProINTA Quintal	31.3	3.80	11.3	0.20	52.7	15.4	30.1	27.4	30.8	28.4	10.7	5.80	7.20	1.90	24.9	11.8
ProINTA Granar	69.5	46.2	16.0	16.0	82.4	58.9	35.0	33.1	97.1	75.2	52.0	39.8	92.1	85.7	63.4	50.7
ProINTA Real	43.4	27.1	9.70	8.02	57.6	34.7	72.5	64.0	63.0	47.2	35.0	30.0	35.0	22.2	45.2	33.3
Thomas Tupungato	17.0	7.80	36.6	23.6	55.1	20.0	60.2	53.4	67.6	66.6	51.6	32.4	89.4	83.5	53.9	41.0
Averages	34.7	19.2	28.6	14.0	34.9	20.8	43.1	37.3	54.8	46.3	30.1	22.1	40.2	31.3	38.0	28.9

LSD necrosis percentage (P=0.05) cultivar =3.67; isolate=2.43; interaction cultivar x isolate=9.71
LSD pycnidial coverage (P=0.05) cultivar=4.54; isolate=3.00; interaction cultivar x isolate=12.02

seedlings (Eyal et al., 1985; Perelló et al., 1991; Ahmed et al., 1995; Ballantyne and Thomson, 1995; Kema et al., 1996a, b). The existence of a cultivar × isolate interaction in adult stage is inferred from a significant interaction term in the ANOVA (Table 3.8). In the adult stage, Kema and Van Silfhout (1997) and Brown et al. (2001) also reported cultivar × isolate interactions.

Multiple linear regression analysis of heading date and plant height on pycnidial coverage showed no relationship between the resistance and any of the morphophysiological traits in seedlings for either of the two field experiments. All cultivars reached the GS 12 at the same time; thus they were inoculated and scored at the same date. In that way no influence of environmental conditions could have affected the level of resistance and its association with the morphophysiological traits. In the adult stage, different results were found in the two experiments (1998 and 2000). In 1999, the same experiment was carried out under controlled conditions and the flag leaf was inoculated at heading. In that way, effects of plant height and heading date on the development of the disease due to environment or epidemiological aspects were minimised. As in this trial there was no influence of any of the morpho-physiological traits on the expression of the disease, it is assumed that associations (negative or positive) found in Exps 1 and 2 can be attributed to variation in weather conditions and not to genetic linkages among those traits.

In 1998, weather was more favourable for the expression of the disease in the early cultivars because precipitation was higher and radiation lower for early cultivars than for late ones (Fig. 3.1). This was especially true when considering a period of 15 days before the beginning of the adult stage evaluations, which started on 28 September and on 15 October for the earliest and latest cultivars, respectively (53.4 and 18.8 mm of precipitation and 3511 and 5127 W m^{-2} d^{-1} of radiation). Negative associations between resistance and days to heading for this year can be attributed to these differences in weather variables. In contrast, in 2000 no negative associations were found between days to heading and the pycnidial coverage percentage. Some positive associations were found at GS 70 and for the AUDPC. Precipitation and temperatures were higher at the beginning of the infection for the latest cultivars.

Adult stage evaluations started on 17 October and 2 November for the earliest and latest cultivars, respectively. Considering a period of 15 days before those dates, mean temperatures were 14.2 and 16.8 °C, precipitation 57.5 and 101.1 mm and mean relative humidity 71.9 and 92.4% for early and late cultivars, causing these significant positive associations between pycnidial coverage and days to heading. Under greenhouse conditions, temperatures from 17 to 25 °C are optimum for disease development (Hess and Shaner 1987, Shaw 1990, Wainshilbaum and Lipps 1991, Magboul et al., 1992, Chungu et al., 2000). High humidity or precipitation and low

radiation at time of infection have also been indicated as conditions conducive to the development of *Mycosphaerella graminicola* (Holmes and Colhoun, 1974; Hess and Shaner, 1987; Shaw and Royle, 1989).

The lack of genetic associations between resistance and heading date agrees with the report by Arama et al. (1999). As they mentioned, when one tries to assess true resistance of a range of cultivars, disease severity should be measured not at the same moment, but at the same stage of development. If, in our experiments, disease development would have been measured at the same time in the adult stage for all cultivars, early cultivars would have been at GS 70 when late ones were at GS 49. That was demonstrated by the overlapping of the last date of evaluation for GS 49 and the first for GS 70 (data not shown). This would have caused high and significant negative associations between earliness and resistance due to differences in leaf age by the time of evaluations and because of differences in the time that the leaves have been exposed to the disease.

Negative associations with plant height were mainly present in 1998 when weather conditions were less conducive to the development of the disease than in 2000. Unconducive conditions and larger distances between leaves in tall cultivars could have reduced the rain-splash dispersal of pycnidiospores causing this negative association. Associations with plant height could also depend on the presence of the teleomorphic state and the importance of the ascospore release during the growth of the wheat crop. The air-borne dispersal of ascospores could reduce the effect of plant height in the expression of the disease. In Argentina, the presence of the teleomorphic state during the whole growing period has been reported (Cordo et al., 1990; Cordo et al., 1999).

In Argentina, information about resistance levels of actual cultivars with different isolates is scarce. The results of this work showed specific interactions between cultivars and isolates, however, some cultivars showed acceptable quantitative levels of resistance with several isolates. Even if these cultivars were susceptible to other isolates, higher levels of resistance could be achieved by intercrossing them. In this germplasm, no genetic associations between earliness, plant height and resistance to septoria tritici blotch are evident. Associations are caused by environmental and epidemiological factors.

CHAPTER 4

Influence of plant height and heading date on the expression of the resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in near isogenic lines of wheat[1]

[1] This chapter has been submitted as:

M.R. Simón, A.J. Worland, P.C. Struik. 2003. Influence of plant height and heading date on the expression of the resistance to septoria tritici blotch (*Mycosphaerella graminicola*) in near isogenic lines of wheat.

Abstract

The effects of plant height and heading date on the expression of the resistance to septoria tritici blotch were investigated in near isogenic lines of wheat differing in dwarfing genes (*Rht*) or in genes encoding for insensitivity to photoperiod (*Ppd*). Crops were inoculated at the 4-leaf stage in two experimental years. Necrosis percentage at boot (GS 49), milk (GS 73) and dough stage (GS 85), area under disease progress curve (AUDPC), plant height and heading date were recorded. The shortest lines, Mercia *Rht*12 and Mercia *Rht*3, showed higher necrosis percentage than Mercia control at all three growth stages and also higher AUDPC values. Mercia lines with gene *Ppd*1, the earliest heading lines, showed lower necrosis values than Mercia control. Cappelle *Rht*3, the shortest of the set of near isogenic Cappelle lines, showed higher necrosis percentage than Cappelle control, whereas Cappelle *Ppd*1 and *Ppd*2 had a lower necrosis percentage than Cappelle control. Multiple linear or non-linear regression models including plant height and heading date accounted for 44.3 to 99.1% of the variation in necrosis percentage and were statistically significant in all stages of both years except for the Mercia set at GS 49 of the experiment in 2000 and for GS 85 of the experiment in 2001. Lower plant height was usually associated with more necrosis and late heading date was not associated or positively associated with more necrosis. When weather variables were included in the models, mean temperature from inoculation to evaluation, humidity and precipitation before inoculation replaced days to heading in most of the stepwise models suggesting that the positive relationship between necrosis and days to heading was caused by environmental conditions. In contrast to literature reports, our data demonstrate that depending on weather conditions positive associations between susceptibility to septoria tritici blotch and heading date can be found. Corrections of disease severity values for heading date and plant height should be done in breeding programmes when selecting for resistance.

Key words: *Triticum aestivum, Mycosphaerella graminicola,* septoria tritici blotch, plant height, heading date, resistance, isogenic lines.

Introduction

Mycosphaerella graminicola (Fuckel) Schroeter, in Cohn (*Septoria tritici* Rob. ex Desm.) is an important disease in many wheat-growing areas of the world (Shipton et al., 1971; King et al., 1983; Eyal et al., 1987). Progress in breeding has been slow due to different factors including a great variability in the pathogen, a certain degree of specificity and the fact that breeding has concentrated on monogenic resistance, which implies specificity to some isolates that is readily broken down.

Furthermore, one of the most confounding factors in selecting for resistance to the septoria tritici blotch could be the reported interaction between resistance and plant height or heading date. Several scientists reported genetic associations between increased disease severity with earliness (Rosielle and Brown, 1979; Eyal, 1981; Camacho Casas et al., 1995) or shortness (Rosielle and Brown, 1979; Baltazar et al., 1990; Camacho Casas et al., 1995). Arama et al. (1999) and Simón et al. (2001b), however, did not detect genetic associations between resistance and plant height or heading date. Van Beuningen and Kohli (1990) indicated that these associations are due to epidemiological factors.

Assuming no genetic associations it is important to know whether plant height or heading date affects the expression of the resistance to septoria tritici blotch due to epidemiological or environmental factors. To obtain this knowledge more precisely, experimentation including genotypes, which are genetically similar except for plant height or heading date, is necessary. Such experimentation would allow to separate genotypic differences from effects due to other factors. It is also necessary to evaluate the disease at the same phenological crop stage in all lines.

The possible association between septoria tritici blotch severity and plant height or heading date is important when setting targets for incorporating genetic resistance to the disease in short or early heading lines. Knowledge about such associations could also contribute to optimising choice and management of cultivars in areas where septoria tritici blotch is a major disease.

The aim of this work was to determine the effect of plant height and heading date on the development of septoria tritici blotch using near isogenic lines of wheat differing in dwarfing genes or insensitivity to photoperiod genes.

Materials and methods

Experimental details

Two field experiments were conducted at the Estación Experimental J. Hirschhorn,

Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina, during 2000 and 2001. Plots were 1 m long by 0.60 m (three rows) wide. Seeds were vernalised for four weeks at 4-8 °C before sowing. Between plots, 3 rows of oat were sown to avoid interplot interference. Plots were sown on 5 July 2000 and 26 June 2001.

Genotypes included

Eight isogenic lines from the English wheat cultivar Mercia (6 with dwarfing genes, *Rht*, coming from different origins and 2 with photoperiod insensitive alleles, *Ppd*) and 9 isogenic lines from the French wheat cultivar Cappelle-Desprez (7 with *Rht* genes and 2 with *Ppd* genes) were sown in a randomised block design with 4 replications in each year. The lines were: Mercia control, Mercia *Rht*1 (from Norin 10), Mercia *Rht*1 (from Saitama 27), Mercia *Rht*2 (from Norin 10), Mercia *Rht*3 (from Tom Thumb), Mercia *Rht*12 (from Karkagi 522), Mercia *Ppd*1 (from Mara), Mercia *Ppd*1 (from Ciano 67), Cappelle-Desprez control, Cappelle-Desprez *Rht*1 (from Norin 10), Cappelle-Desprez *Rht*1 (from Saitama 27), Cappelle-Desprez *Rht*1 (from Bezostaya), Cappelle-Desprez *Rht*2 (from Ai-bian), Cappelle-Desprez *Rht*2 (from Norin 10), Cappelle-Desprez *Rht*3 (from Tom Thumb), Cappelle-Desprez *Ppd*1 (from Mara) and Cappelle-Desprez *Ppd*2 (from Chinese Spring).

Inoculations

Inoculations were done with a mix of isolates (FALP 3096; FALP 4396; FALP 3398) in both years. The isolates were grown on malt extract agar at 19 °C with 12 h alternating light and dark cycles. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in de-ionised water. The conidial suspension was adjusted to 3×10^6 spores ml^{-1} and applied at the 4-leaf stage in both years. Inoculum was applied at an early stage of development to simulate a natural infection. After inoculation, plants were kept moist by sprinkling water several times a day during a period of 3 days.

Data collection

Necrosis percentage was visually estimated on the upper three leaves of 10-15 plants from the central row of each plot for each replication when each line reached the following growth stages (Zadoks et al., 1974): GS 49 (booting, first awn visible); GS 73 (early milk stage) and GS 85 (soft dough stage). Height from the soil surface to the flag leaf; height from the soil surface to the tip of the tallest spike (total plant height) at dough stage and days to 50% heading were also assessed on 10-15 plants for each plot and each replication. Although height to flag leaf was used in the further analysis, total

height was also measured to determine the real range in plant height of the lines. Area under disease progress curve (AUDPC) for each plot was calculated to summarise the progress of the disease, according to the formula of Shaner and Finney (1977).

Data analysis

Each isogenic data set (derived from Mercia or Cappelle-Desprez) was analysed separately by ANOVA for randomised block designs in a combined analysis for both years at each growth stage and for the AUDPC values.

Multiple linear and non-linear regression analysis considering necrosis percentage as dependent variable and days to heading and height to flag leaf as independent variables were done to determine the percentage of the variation in necrosis accounted for by those morphophysiological traits. Height to flag leaf was used instead of total plant height considering that septoria tritici blotch progresses up to the flag leaf in our wheat growing area.

A stepwise multiple linear regression analysis including weather variables together with height to flag leaf and days to heading was also performed to assess whether different weather conditions can modify the expression of the disease in lines with different morphophysiological characteristics. Necrosis percentage was the dependent variable and the independent variables were: mean temperature (°C), sum of precipitation (mm), number of days with precipitation of at least 1 mm; mean relative humidity (%) and mean radiation (W m^{-2}) for periods of 3, 7, 15, or 30 days prior to the date of evaluation of each line for each growth stage (indicated as T_3, T_7, T_{15}, T_{30}, P_3, P_7, P_{15}, P_{30}, Dp_3, Dp_7, Dp_{15}, Dp_{30}, H_3, H_7, H_{15}, H_{30} and R_3, R_7, R_{15}, R_{30}, respectively). Except for sum of precipitation and number of days with precipitation (because they are affected by the duration of the period from inoculation to evaluation) also averages from inoculation to evaluation were considered for the other weather variables (indicated as T_i, H_i, R_i).

Results

Mercia data set

The ANOVA for the near isogenic lines from Mercia showed statistically significant differences for necrosis percentage at GS 49, GS 73 and GS 85, for the AUDPC, for plant height (to flag leaf and total) and for days to heading between lines and years. The interaction line × year was significant for necrosis percentage at GS 49 and GS 85 and for plant height (to flag leaf and total) and days to heading (Table 4.1). Average for necrosis percentage for the three growth stages and for the AUDPC was higher in

Table 4.1. ANOVA for necrotic percentage caused by *Mycosphaerella graminicola* at three growth stages, plant height to flag leaf and heading date in 8 near isogenic lines of the Merciawheat cultivar, differing in plant height or days to heading.

Source of variation	df	Mean squares						
		Necrosis percentage			AUDPC	Height to flag leaf	Height total	Days to heading
		GS 49	GS 73	GS 85				
Line	7	1099.7 (P=0.000)§	1195.1 (P=0.000)	397.4 (P=0.000)	1473430 (P=0.000)	2393.3 (P=0.0000)	3748.1 (P=0.000)	835.6 (P=0.000)
Year	1	7543.5 (P=0.000)	20592.3 (P=0.000)	6136.6 (P=0.000)	20683200 (P=0.000)	2074.8 (P=0.0000)	3084.4 (P=0.000)	1198.9 (P=0.000)
Line x year	7	233.4 (P=0.000)	90.7 (P=0.525)	215.0 (P=0.000)	32881 (P=0.840)	59.4 (P=0.0021)	106.3 (P=0.000)	4.35 (P=0.000)
Error	45	48.6	102.3	28.9	68427	15.2	20.2	9.19

§P>F

Table 4.2. Means for necrosis percentage caused by *Mycosphaerella graminicola* at three growth stages in 8 isogenic lines of the Mercia wheat cultivar (coming from different origins) differing in plant height or days to heading.

	Necrosis (%)			AUDPC	Height to flag leaf (cm)	Total height (cm)	Days to heading
Line	GS 49	GS 73	GS 85				
Mercia control	15.2 c	61.7 b	88.2 b	1977 b	64.5 d	81.2 d	129.4 bcd
Mercia *Rht* 1 (Norin 10)	12.7 bc	55.5 b	82.1 ab	1885 b	53.7 c	69.4 c	126.6 b
Mercia *Rht* 1 (Saitama 27)	15.0 c	57.4 b	83.7 ab	1963 b	66.7 d	85.2 d	126.9 b
Mercia *Rht* 2 (Norin 10)	17.4 cd	60.3 b	89.0 b	2100 b	47.7 b	62.7 b	128.1 bc
Mercia *Rht* 3 (Tom Thumb)	22.6 d	72.8 c	95.6 c	2456 c	24.8 a	33.8 a	132.1 d
Mercia *Rht* 12 (Karkagi 522)	42.9 e	73.9 c	100.0 c	2763 d	25.8 a	32.9 a	130.9 cd
Mercia *Ppd* 1 (Mara)	5.4 a	38.7 a	80.9 a	1448 a	64.2 d	82.0 d	106.5 a
Mercia *Ppd* 1 (Ciano 67)	6.9 ab	44.3 a	81.4 a	1584 a	63.4 d	82.3 d	108.1 a
Years							
2000	6.4 a	40.9 a	77.8 a	1453 a	57.0 a	73.1 a	119.2 a
2001	28.1 b	76.0 b	97.4 b	2590 b	45.7 b	59.3 b	127.9 b

2001 than in 2000 at the three growth stages. Plant height (to flag leaf and total) was lower and days to heading longer in 2001 than in 2000 due to the earlier sowing in 2001. For the average of the two years, genes *Rht*1 (Norin 10), *Rht*2, *Rht*3 and *Rht*12 significantly reduced plant height (to flag leaf and total). Gene *Rht*1 coming from Saitama 27, which is known to be a less potent allele than *Rht*1 (Norin 10) (C. Law, 2002, personal communication) did not have effects on the height of Mercia control. *Ppd*1 (Mara) and *Ppd*1 (Ciano 67) significantly decreased the days to heading compared to the Mercia control. The shortest lines Mercia *Rht*12 and Mercia *Rht*3 showed higher necrosis percentage at the three growth stages and higher AUDPC values than Mercia control. Mercia *Ppd*1 and *Ppd*2 showed lower necrosis values at the three growth stages and lower AUDPC values than Mercia control (Table 4.2).

The significant interaction line × year for days to heading was due to the fact that lines with *Ppd*1 and *Ppd*2 were insensitive to photoperiod and thus had similar days to heading in both years whereas the other lines were later in 2001 than in 2000 due to the earlier sowing. The interaction line × year for plant height was due to the fact that the shortest lines (Mercia *Rht*3 and Mercia *Rht*12) had similar values in both years whereas the other lines were shorter in 2001 (data not shown). The significant interaction line × year for necrosis percentage at GS 49 was due to the fact that in 2000 necrosis was low and similar in all cultivars except for Mercia *Rht*12 which showed higher values. However in 2001, the earliest heading lines *Ppd*1 (Mara) and *Ppd*1 (Ciano 67) showed lower values than the control, whereas Mercia *Rht*12 and Mercia *Rht*3 showed higher values than the control (Fig. 4.1A). At GS 85, the interaction line × year can be explained by the fact that in 2001 differences between lines were small because all lines reached high and similar disease severity, whereas in 2000 there was discrimination between them, with lines Mercia *Ppd*1 (Mara) and Mercia *Ppd*1 (Ciano 67) showing lower necrosis percentages than the control and Mercia *Rht*3 and Mercia *Rht*12 showing higher values than the control (Fig. 4.1C).

Cappelle-Desprez data set

For the Cappelle-Desprez set there were significant differences between lines and years for necrosis percentage at the three growth stages, for the AUDPC values and for plant height and days to heading. The interaction line × year was also significant for necrosis percentage at the three growth stages (Table 4.3). All *Rht* genes significantly reduced plant height compared to the control and *Ppd* genes shortened the period to heading. On average, lines Cappelle-Desprez *Ppd*1 showed lower disease severity than the control at the three growth stages and Cappelle-Desprez *Ppd*2 at GS 73 and GS 85. Cappelle-Desprez *Rht*3, the shortest line, showed higher necrosis percentages than the control at GS 49 and GS 73 and Cappelle-Desprez *Rth*2 (Norin) was higher at GS 49.

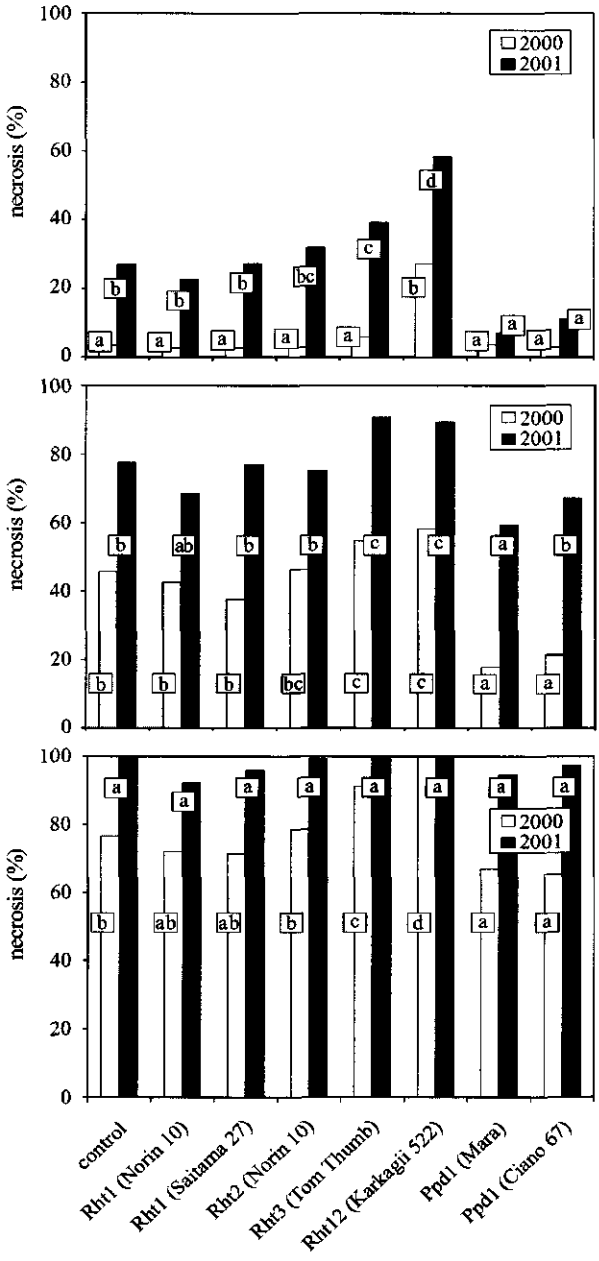

Fig. 4.1. Interactions line × year for necrosis percentage caused by *Mycosphaerella graminicola* in 8 near isogenic lines of the wheat cultivar Mercia, differing in plant height and heading date at (top) boot stage (GS 49, Zadoks et al., 1974), (middle) milk stage (GS 73), (bottom) dough stage (GS 85). Experiments were carried out in 2000 and 2001.

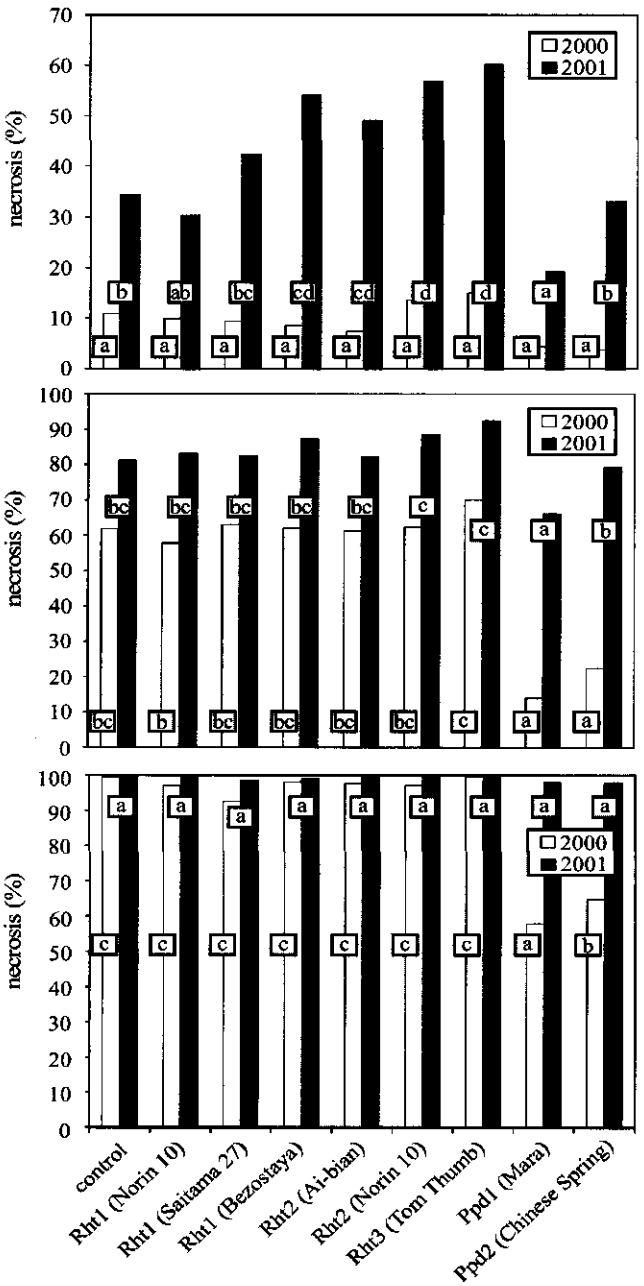

Fig. 4.2. Interactions line × year for necrosis percentage caused by *Mycosphaerella graminicola* in 9 near isogenic lines of the wheat cultivar Cappelle-Desprez, differing in plant height and heading date at (top) boot stage (GS 49, Zadoks et al., 1974), (middle) milk stage (GS 73, (bottom) dough stage (GS 85). Experiments were carried out in 2000 and 2001.

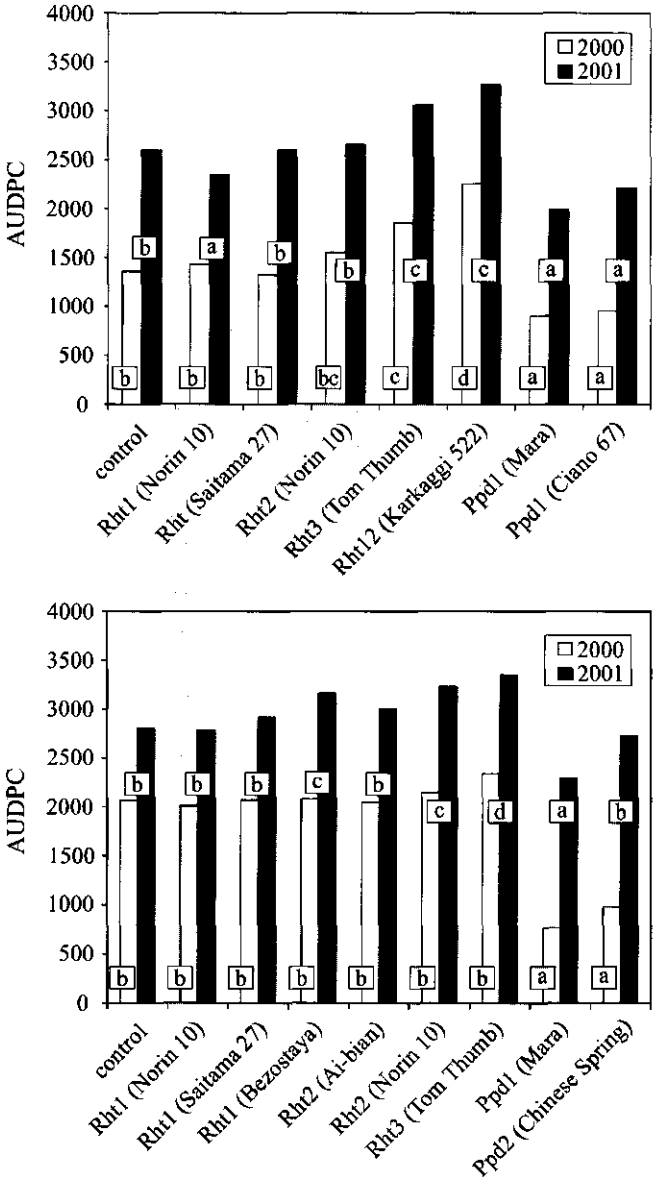

Fig. 4.3. Interactions line × year for the area under disease progress curve (AUDPC) caused by *Mycospharella graminicola* in (top) 8 isogenic lines of the wheat cultivar Mercia, (bottom) 9 near isogenic lines of the wheat cultivar Cappelle-Desprez. Experiments were carried out in 2000 and 2001.

For the AUDPC, line *Rht*3 showed higher values than the control and *Ppd*1 and *Ppd*2 showed lower values (Table 4.4). For each of the three growth stages there was a higher disease severity in 2001 than in 2000.

The interactions line × year at GS 49 can be explained by low and similar severity values for all the lines in 2000 but not in 2001. In 2001, line Cappelle-Desprez *Ppd*1 showed lower necrosis percentage than the control and lines *Rht*1 (Bezostaya), *Rht*2 (Ai-bian), *Rht*2 (Norin 10) and *Rht*3 higher values than the control. At GS 73, *Ppd*1 and *Ppd*2 showed significantly lower values than the control in 2000, whereas in 2001 only *Ppd*1 was lower than the control. At GS 85, only *Ppd*1 and *Ppd*2 in 2000 showed lower values than the control (Figs. 4.2A, B, C). The interaction line × year for the AUDPC values was due to the fact that in 2000 there was less discrimination between lines and none of the lines had significantly higher values than Cappelle-Desprez control, whereas in 2001 *Rht*1 (Bezostaya), *Rht*2 (Norin 10) and *Rht*3 (Tom Thumb) had higher necrosis percentage than the control (Fig. 4.3B).

Multiple regression models

Linear multiple regression models including plant height and days to heading as independent variables and necrosis percentage as dependent variable showed that for the set of Mercia plant height to flag leaf was negatively associated with necrosis at GS 73 in both years, at GS 49 in 2001 and at GS 85 in 2000 ($P<0.05$). Heading date was positively associated with necrosis (at $P<0.10$) at GS 49 in 2001 and at GS 73 and GS 85 in 2000. Models including both independent variables accounted for 35.3 to 98.3% of the variation in necrosis percentage, being significant for all cases except at GS 49 in 2000 and at GS 85 in 2001 (Table 4.5).

A large set of non-linear regression models were also tested. However, only for GS 49 in 2000, a model with better probability of the R^2 values than for multiple linear regression was identified.

The complicated predictive model was:

$$\text{necrosis (\%)} = -460.4\ (P=0.002) + 16.99\ (P=0.002)\ x_2 - 0.1314\ (P=0.001)\ x_1x_2 + 0.00023\ x_1^3\ (P=0.001) - 0.000080\ (P=0.023)\ x_2^3;$$
$$R^2=98.92\%\ (P=0.003),$$

with x_1 being days to heading and x_2 plant height.

For the Cappelle-Desprez set, plant height was significantly negatively associated with necrosis percentage at GS 73 in both years ($P<0.10$) and at GS 49 in 2001 ($P<0.05$). Days to heading were significantly positively associated with necrosis percentage at GS 73 and GS 85 in both years and at GS 49 in 2001 ($P<0.05$). Models accounted for 66.1% to 99.1% of the variation in necrosis percentage, being significant for both years and at all three growth stages (Table 4.6).

Table 4.3. ANOVA for necrotic percentage caused by *Mycosphaerella graminicola* at three growth stages, AUDPC, plant height to flag leaf, total plant height and heading date in 9 near isogenic lines of the wheat cultivar Cappelle-Desprez differing in plant height or days to heading.

Source of variation	df	Mean squares						
		Necrosis percentage			AUDPC	Height to flag leaf	Height (total)	Days to heading
		GS 49	GS 73	GS 85				
Line	7	553.6 (P=0.0000)[a]	1403.6 (P=0.0000)	570.2 (P=0.0000)	1432750 (P=0.0000)	1528.6 (P=0.0000)	2070.5 (P=0.0000)	764.4 (P=0.0000)
Year	1	19367.7 (P=0.0000)	16379.3 (P=0.0000)	1723.8 (P=0.0000)	21668700 (P=0.0000)	2389.7 (P=0.0000)	5038.4 (P=0.0000)	2167.0 (P=0.0000)
Line × year	7	268.7 (P=0.0024)	392.6 (P=0.0000)	472.0 (P=0.0000)	2174690 (0.0006)	27.3 (P=0.3507)	74.9 (P=0.0401)	41.26 (P=0.0747)
Error	45	75.6	41.0	18.3	51169.8	23.9	33.6	21.33

[a] P>F

Table 4.4. Means for necrotic percentage caused by *Mycosphaerella graminicola* at three growth stages in 9 isogenic lines of the wheat cultivar Cappelle-Desprez differing in plant height or days to heading.

Line	Necrosis (%)			AUDPC	Height to flag leaf (cm)	Total height (cm)	Days to heading
	GS 49	GS 73	GS 85				
Cappelle control	22.6 bcd	71.6 c	99.7 cd	2526 d	70.4 e	89.5 e	135.1 cd
Cappelle *Rht*1 (Norin 10)	20.1 abc	70.5 c	98.3 cd	2265 cd	60.4 d	77.6 d	130.7 c
Cappelle *Rht*1 (Saitama 27)	25.8 bcd	72.8 c	95.5 c	2495 d	59.8 d	75.8 d	132.6 cd
Cappelle *Rht*1 (Bezostaya)	31.3 def	74.7 c	98.6 cd	2624 de	46.7 b	60.8 b	135.0 cd
Cappelle *Rht*2 (Ai-bian)	28.2 cde	71.7 c	98.9 cd	2527 d	52.3 c	68.1 c	134.2 cd
Cappelle *Rht*2 (Norin 10)	35.1 ef	75.5 cd	98.6 cd	2688 de	56.5 cd	73.0 cd	136.1 d
Cappelle *Rht*3 (Tom Thumb)	37.5 f	81.2 d	99.8 d	2843 e	29.1 a	39.0 a	136.4 d
Cappelle *Ppd*1 (Mara)	11.8 a	39.9 a	77.9 a	1856 b	70.4 e	87.3 e	108.7 a
Cappelle *Ppd*2 (Ciano 67)	18.3 ab	50.8 b	81.4 b	1531 a	72.7 e	88.3 e	117.4 b
Years							
2000	9 .23 a	52.40 a	89.42 a	1824 a	63.35 a	81.62 a	124.1 a
2001	42.03 b	82.56 b	99.21 b	2921 b	51.82 b	64.89 b	135.1 b

Means followed by the same letter in the same column are not statistically significant, LSD (P=0.05).

Table 4.5. Multiple linear regression of days to heading and plant height to flag leaf (independent variables) on necrosis percentage (dependent variable) caused by *Mycosphaerella graminicola* in 8 near isogenic lines of the wheat cultivar Mercia at three growth stages in two years.

Growth stage/year	Estimates			R^2 model (%)
	Constant	Height	Days to heading	
GS 49				
Year 2000	8.45 (0.883)*	–0.26 (0.182)	0.11 (0.799)	44.27 (0.232)
Year 2001	–16.22 (0.706)	–0.62 (0.034)	0.57 (0.084)	86.22 (0.007)
GS 73				
Year 2000	–84.35 (0.003)	–0.34(0.001)	1.20 (0.000)	98.28 (0.000)
Year 2001	49.91 (0.141)	–0.42 (0.039)	0.35 (0.122)	83.59 (0.011)
GS 85				
Year 2000	34.37 (0.348)	–0.43(0.009)	0.57 (0.071)	89.72 (0.003)
Year 2001	101.23 (0.001)	–0.11(0.248)	0.0082 (0.939)	35.26 (0.337)

* Probability, t-test.

Table 4.6. Multiple linear regression of days to heading and plant height to flag leaf (independent variables) on necrosis percentage (dependent variable) caused by *Mycosphaerella graminicola* in 9 near isogenic lines of the wheat cultivar Cappelle-Desprez at three growth stages for two years.

Growth stage/ year	Estimates			R^2 model
	Constant	Height	Days to heading	
GS 49				
Year 2000	–9.31 (0.591)*	–0.09 (0.254)	0.20 (0.109)	67.49 (0.034)
Year 2001	–41.56 (0.326)	–0.51 (0.030)	0.81 (0.02)	88.65 (0.001)
GS 73				
Year 2000	–140.37 (0.000)	–0.24 (0.029)	1.68 (0.000)	98.53 (0.000)
Year 2001	14.82 (0.441)	–0.18 (0.073)	0.57 (0.002)	91.68 (0.001)
GS 85				
Year 2000	–92.58 (0.000)	–0.06 (0.317)	1.50 (0.000)	99.13 (0.000)
Year 2001	88.73 (0.000)	0.003 (0.894)	0.077 (0.029)	66.07 (0.039)

* Probability, t-test.

No non-linear-regression models with higher probabilities of the R^2 values than for multiple linear models could be identified.

Figure 4.4 (A, B, C and D) indicates mean daily temperature (°C), daily precipitation (mm), mean daily relative humidity (%) and mean daily global radiation (W m^{-2}) for 2000 and 2001. Mean temperatures for the period from inoculation to evaluation varied from 14.1 to 15.8; 15.8 to 17.4 and 16.8 to 18.4 °C for early and late Mercia lines at GS 49, GS 73 and GS 85, respectively in 2000 and from 15.2 to 20.5; 16.8 to 17.9 and 18.2 to 19.9 °C at GS 49; GS 73 and GS 85 in 2001. Precipitation 15 days before evaluation which accounted for part of the variation in necrosis percentage at GS 73 in 2000 ranged from 25.5 to 48.6 mm for early and late cultivars in 2000. For the Cappelle-Desprez lines, mean temperature ranged from 14.1 to 16.1 (evaluation at GS 49), 14.1 to 16.1 (GS 73) and 16.8 to 18.9 °C (GS 85) for early and late lines in 2000 and from 14.4 to 17.6 (evaluation at GS 49), 17.1 to 18 (GS 73) and 18.0 to 19.4 °C (GS 85) in 2001. Precipitation 15 days before evaluation, which accounted for part of the variation in necrosis percentage at GS 73 in 2001 varied from 70 to 153 mm for late and early cultivars, respectively.

Multiple linear regression using weather data as independent variables and necrosis as dependent variable yielded several statistically significant models. Usually the best predictors included temperature and humidity from inoculation to evaluation or for a shorter period before inoculation. In some cases, single linear regression on weather data already yielded highly significant models. Humidity 15 days before evaluation accounted for 75% of the variation in necrosis percentage among lines of Cappelle-Desprez at GS 49 in 2000, whereas the temperature from inoculation to evaluation accounted for 98.5 and 76.6% of the variation in necrosis of Cappelle-Desprez lines at the dough stage in 2001 and 2000, respectively (Table 4.8).

When environmental variables were included in the multiple linear models together with height to flag leaf and heading date to account for variation in necrosis percentage, the stepwise multiple linear regression analysis determined that for the Mercia set plant height to flag leaf remained negatively associated with necrosis for most of the models, whereas days to heading was replaced by environmental variables. These variables were mainly mean temperatures in the period from inoculation to evaluation, or temperature or rainfall mainly during the 15 days before evaluations (Table 4.7).

For the Cappelle-Desprez set, plant height to flag leaf consistently was also one of the most important variables in predicting variation in necrosis percentage in several of the models. Days to heading was replaced by environmental variables such as temperature from inoculation to evaluation or some days before evaluations or relative humidity. For GS 73 in 2001, also a negative association with precipitation 30 days

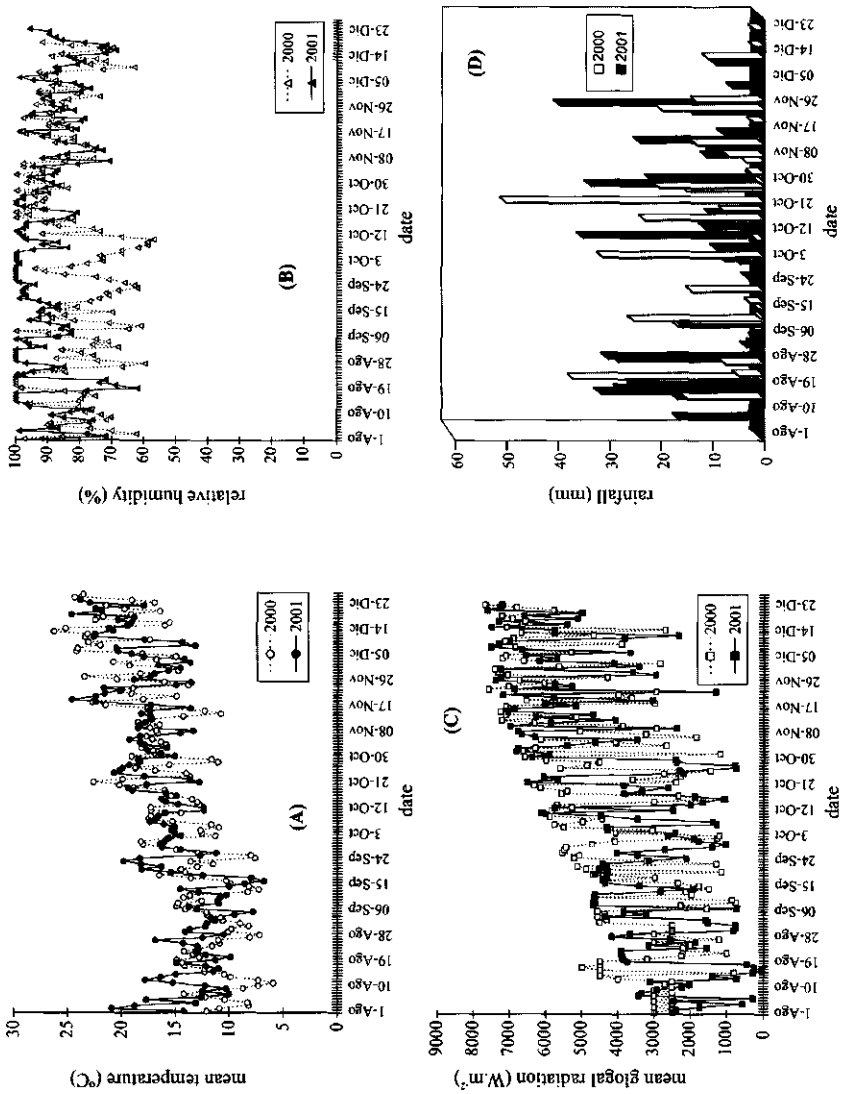

Fig. 4.4. Weather variables in the growing wheat period after inoculation with *Mycosphaerella graminicola* in two years. (A) Mean daily temperature; (B) mean relative humidity; (C) mean global radiation; (D) rainfall.

before evaluation was shown when precipitation 30 days before evaluation varied from 70 to 153 mm for early and late cultivars, respectively (Table 4.8).

Discussion

The increase in the severity of the disease in 2001 can be mainly attributed to the inoculation in the previous year and proximity to a non-till wheat crop. Although the necrosis percentage of the near isogenic lines followed similar patterns in both years, the interaction line × year for both sets of isogenic lines was mainly due to a higher inoculum pressure in 2001 than in 2000. This resulted in a low discrimination between lines at boot stage in 2000, due to low necrosis percentages for most of the lines and no discrimination at dough stage in 2001, due to very high values for all the lines. For the Mercia set, differences between lines in plant height were higher than for the Cappelle-Desprez set, due to the presence of the *Rht*12 gene in one of the isogenic lines that caused an important height reduction. *Rht*3 also greatly reduced plant height in both sets. Lines with higher necrosis percentages were generally those carrying

Table 4.7. Best models explaining variation in necrosis caused by *Mycosphaerella graminicola* (dependent variables) using plant height, days to heading and some weather variables as independent variables (by stepwise multiple regression) in 8 near isogenic lines of the Mercia wheat cultivar.

Growth stage/year	Model	R^2 (%)
GS 49		
2000	22.87 (0.030)* – 0.288 (0.070) Hfl	44.27 (0.232)
2001	–44.56 (0.270) – 0.54 (0.030) Hfl + 5.96 (0.020) Ti	91.50 (0.002)
GS 73		
2000	–111.87 (0.002) + 0.18(0.090) P15 + 9.3 (0.000) T15 – 0.39 (0.000) Hfl	99.50 (0.000)
2001	101.66 (0.000) – 0.56 (0.011) Hfl	68.74 (0.011)
GS 85		
2000	–29.83 (0.580) + 7.32 (0.040) Ti – 0.43 (0.006) Hfl	91.71 (0.002)
2001	101.23 (0.001) + 0.008 (0.939) Hd – 0.11 (0.248) Hfl	92.94 (0.000)

* Probability, t-test.

Hfl is plant height to flag leaf; Ti is temperature from inoculation to evaluation; P15 is rainfall during the 15 days before evaluation; T15 is temperature during the 15 days before evaluation; Hd is heading date.

Table 4.8. Best models explaining variation in necrosis caused by *Septoria tritici* (dependent variables) using plant height, days to heading and some weather variables as independent variables (by stepwise multiple regression) in 9 isogenic lines of the Cappelle-Desprez wheat cultivar.

Growth stage/year	Model	R^2 (%)
GS 49		
2000	−69.83 (0.050)* + 0.92 (0.002) H15	75.19 (0.002)
2001	−53.46 (0.220) − 0.53 (0.016) Hfl + 7.43 (0.010) Ti	91.50 (0.002)
GS 73		
2000	− 325.61 (0.000) − 0.25 (0.011) Hfl + 22.80 (0.000) Ti	98.97 (0.000)
2001	− 73.66 (0.210) − 0.16 (0.040) Hfl − 0.15 (0.002) P30 + 10.08 (0.016) T30	96.95 (0.000)
GS 85		
2000	−262.91 (0.000) + 19.18 (0.000) Ti	98.46 (0.000)
2001	62.18 (0.000) + 1.97 (0.002) Ti	76.58 (0.002)

* Probability t-test.

H15 is humidity during the 15 days before evaluation; Hfl is plant height to flag leaf; Ti is temperature from inoculation to evaluation; P30 is precipitation during 30 days before evaluation; T30 is temperature during 30 days before evaluation.

these genes. For the AUDPC values, interaction line × year was only evident for the Cappelle-Desprez set. This can also be explained by the fact that under low inoculum pressure (in 2000) discrimination between lines was lower than in 2001.

Multiple regression models showed that, in general, plant height was negatively associated with resistance; heading date was positively associated. However, when weather variables were included in stepwise regression models together with the morphophysiological traits, plant height to flag leaf remained negatively associated whereas heading date was replaced by relevant weather variables such as temperature, rainfall and relative humidity in different periods prior to evaluations. This suggests that the positive association between heading date and resistance was because of weather conditions in these years being conducive to the development of septoria tritici blotch, especially in the lines with late heading. Several investigations have found negative associations between necrosis with plant height and heading date, attributed in some cases to genetic linkages and in some other to epidemiological or environmental factors (Rosielle and Brown, 1979; Eyal et al., 1987; Camacho Casas et al., 1995; Baltazar et al., 1990; Van Beuningen and Kohli, 1990). Our previous studies and investigations of other researchers indicated no genetic associations within a wide set of cultivars for those traits (Arama et al., 1999; Simón et al., 2001b, 2002). This

work shows that associations are due to epidemiological or environmental factors. Reductions in plant height are generally associated with increases in necrosis percentages due to the fact that shorter distances between leaf layers make inoculum transference easier.

Weather variables, depending on the growing region, year, or sowing date, could be also neutral or favourable for the development of septoria tritici blotch in early heading cultivars, as has been found in other studies (Simón et al., 2001b, 2002, 2003). Without the intention of making a predictive model we demonstrated that some weather conditions were more conducive to septoria tritici blotch in the latest heading near isogenic lines which had higher necrosis percentage than the earliest heading near isogenic lines. Under greenhouse conditions, temperatures from 17 to 25 °C are optimum for disease development (Hess and Shaner, 1987; Shaw, 1990; Wainshilbaum and Lipps, 1991; Magboul, 1992; Chungu et al., 2000). In this investigation, temperature was the main environmental factor positively associated with necrosis, because especially at GS 49 and GS 75 for these growing seasons, mean temperatures were lower for early heading cultivars than for late heading cultivars. Precipitation, relative humidity and radiation were more similar for all cultivars. At GS 73 for the Cappelle-Desprez set, negative associations were found with precipitation 30 days prior to evaluation probably due to the fact that the rainfall in this phase was too heavy for normal transport of spores.

Evaluating the disease at the same crop phenological stage is crucial. When the disease is assessed at the same chronological time, leaves of late cultivars are younger and less exposed to the pathogen. Moreover, for *Septoria* spp. resistance decreases with leaf age, and the older lower leaves are more susceptible than the younger upper leaves (Jonsson, 1991). Due to this effect, late maturing germplasm is often considered more resistant than early maturing lines.

In this research, strong associations with plant height were only found in very short wheats (in general with differences in height to flag leaf higher than 38 cm compared to the control) indicating that moderately short wheats are not necessarily more susceptible to septoria tritici blotch than the taller ones. Considering that a wide spectrum of wheat cultivars fell far from plant height values of the shortest lines used in this study, attention should be paid to the choice of varieties in areas where septoria tritici blotch is a major disease. Knowing the climatic characteristics of the wheat growing region it is possible to adjust sowing dates and cultivar choice (based on heading date) to avoid periods with weather conditions predisposing to the disease during critical phases of the wheat crop. In breeding programmes, disease severity values for septoria tritici blotch should be corrected for plant height and heading date in each particular situation to determine true resistance values.

CHAPTER 5

Influence of nitrogen supply on the susceptibility of wheat to septoria tritici blotch (*Mycosphaerella graminicola*)[1]

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Abstract

Nitrogen (N) fertilisation is required for achieving high yields in wheat (*Triticum aestivum* L.) but may enhance the development of septoria tritici blotch [*Mycosphaerella graminicola* (Fuckel) Schroeter, in Cohn]. A study was undertaken to investigate how N supply influences the AUDPC (area under disease progress curve). Two field experiments were carried out in a Typical Argiudol in 1996 and 1997. Six cultivars were grown at two N treatments (0 and 100-150 kg N ha^{-1}) in a split plot design. Percentage of necrosis (disease severity) of the two upper leaves of each treatment was evaluated three times during the growing season. Plant height and heading date were also scored and related with the resistance level. In 1996, with weather conditions conducive to the disease, AUDPC values were higher in the fertilised treatment. In 1997, with insufficient rain immediately after inoculation, the disease only progressed faster under N fertilisation in the flag leaf, which was exposed to conducive environmental conditions from its appearance. The effect of N fertilisation was influenced by the cultivar characteristics, climatic, and agronomic conditions. Knowledge that N fertilisation promotes the development of septoria tritici blotch in conducive conditions will be useful for deciding management strategies of the cultivars and for optimising conditions for the selection in breeding programmes.

Key words: *Mycosphaerella graminicola*, septoria tritici blotch, N fertilisation, *Triticum aestivum*, wheat.

Introduction

Leaf blotch, caused by *Mycosphaerella graminicola* (Fuckel) Schroeter, in Cohn (anamorph *Septoria tritici* Rob. ex Desm.), is an important disease in many wheat-producing areas of the world, and can cause significant yield losses (Eyal et al., 1985, 1987). It is a major problem in regions characterised by a temperate, high rainfall environment during the growing season (Holmes and Cohloun, 1974; Eyal et al., 1987).

Several physiological and environmental factors influence the expression of resistance to septoria tritici blotch, including crop growth stage (Holmes and Cohloun, 1974; Tavella, 1978; Wainshilbaum and Lipps, 1991; Arama, 1996; Kema and Van Silfhout, 1997; Simón and Cordo, 1999), air temperature (Holmes and Cohloun, 1974; Wainshilbaum and Lipps, 1991), relative humidity (Holmes and Cohloun, 1974) and rainfall (Thomas et al., 1989).

Agronomic practices also influence septoria tritici blotch severity by modifying the microclimate within the crop canopy (Shaw and Royle, 1989) or the nitrogen concentration in the leaves (Leitch and Jenkins, 1995), but the magnitude and direction of these effects are inconsistent. Increased N-fertility has been reported to increase the severity of the disease (Gheorghies, 1974; Prew et al., 1983; Broscious et al., 1985; Howard et al., 1994; Leitch and Jenkins, 1995). Hayden et al. (1994) found higher severities at higher N rates in a greenhouse study but not in the field. Johnston et al. (1979) reported a decrease in the severity of the disease with increased N in one year of their experiments. Tompkins et al. (1993) in no-till wheat and Arama (1996) found variable results within their experiments. The incidence of several wheat diseases depends on the form in which N is applied (Huber and Watson, 1974). Thus, although N seems to influence the severity of the septoria tritici blotch, there is no clear correlation as to how the influence is expressed since the conditions of the experiments have been very different. Therefore, N effects on septoria tritici blotch could have been interacting with climate, soil type, nitrogen dose applied, canopy structure, previous crop, time of application, cultivar resistance and available natural inoculum (Johnston et al., 1979; Prew et al., 1983; Hayden et al., 1994; Leitch and Jenkins, 1995; Arama, 1996). It also has been suggested that inconsistencies of septoria tritici blotch response to N-fertilisation may be due in part to environmental factors such as ozone (Tiedemann, 1996).

The aim of this work was to investigate the influence of N fertilisation on the progress of septoria tritici blotch and to evaluate how environmental conditions can interact with this effect.

Materials and methods

Two experiments were conducted at the Estación Experimental J. Hirschhorn, Facultad de Ciencias Agrarias, Universidad Nacional de La Plata, Argentina, during 1996 and 1997. Weather data (air temperature, humidity and rainfall) were recorded at the Meteorological Station situated 100 m from the experiment. These weather data are indicated in Fig. 5.1 for both years. The trials were sown on 17 July 1996 and 5 August 1997, under conventional tillage. The soil was a Typical Argiudol. Analysis of the soil samples (top 0.20 m) indicated the following values: organic matter=3%; N=0.17%; P=7 mg kg^{-1} and pH=5.9. The experimental design was a split-plot with four replications. Within each year, main plots were the nitrogen treatments, 0 and 100 kg N ha^{-1} as urea at sowing in 1996, and 0 and 100 kg ha^{-1} as urea at sowing plus 50 kg ha^{-1} as urea at Growth Stage (GS) 30, (Zadoks et al., 1974) in 1997. Subplots were the registered cultivars: Buck Ombú (B. Ombú), Don Ernesto INTA (D. Ernesto), Klein Centauro (K. Centauro), Klein Dragón (K. Dragón), ProINTA Federal (P.I. Federal) and ProINTA Isla Verde (P.I. I. Verde). These cultivars were known to differ in their resistance to septoria tritici blotch according to the information provided by their respective breeders. Each subplot was 6.3 m^2 (4.5 m long by 1.4 m wide). The entire experiment was fertilised with 50 kg P_2O_5 ha^{-1} as calcium triple superphosphate at the time of sowing. A virulent isolate (FALP 3096, Facultad de Ciencias Agrarias de La Plata, Argentina) of *Mycosphaerella graminicola* was used to prepare the inoculum. The isolate was grown on malt extract agar at 19 °C with 12 h alternating light and dark cycles. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in de-ionised water. The spore concentration was measured with a Newbauer hemacytometer. The conidial suspension was adjusted to 5×10^6 spores ml^{-1} and 0.5 ml of Tween 20 per liter was added as a surfactant. Two inoculations were carried out (at the beginning of tillering, GS 21, and at the beginning of shoot development, GS 31, in both years) which coincided with the stages when the disease typically was first evident under natural conditions in the region. Inoculations were performed at the beginning and at the end of September 1996 and in the middle of September and the beginning of October in 1997. Plants were sprayed with the inoculum suspension until runoff. After inoculation, plants were kept moist by spraying with water several times a day for a period of 3 days. The trial was sprayed with Plantvax (oxicarboxin, 5-6 dihydro-2 methyl-N-phenyl-1,4-oxathiin-3-carbozamide 4,4-dioxide), a specific selective fungicide for the prevention of rust diseases, when the first symptoms of leaf rust (caused by *Puccinia triticina* Eriks) appeared.

In 1996, necrosis percentage on the flag leaf (FL) and the leaf below the flag

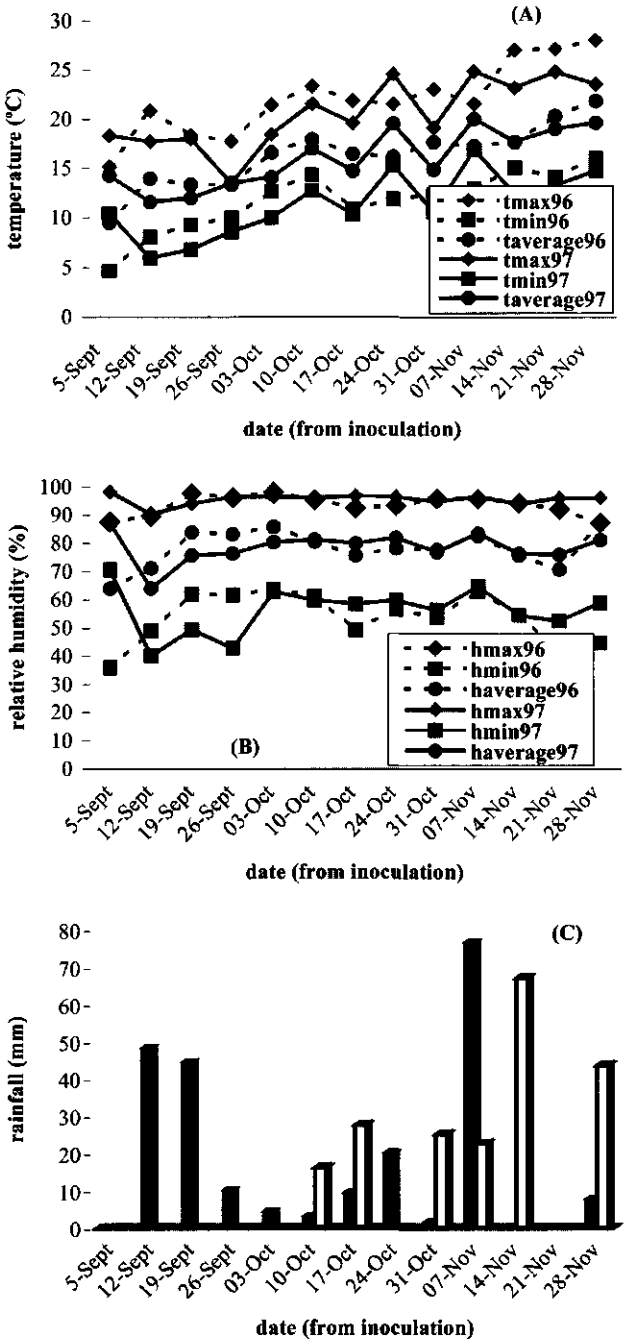

Fig. 5.1. Weather data for two growing seasons (1996 and 1997); (A) average of weekly temperatures; (B) Average of weekly relative humidity; (C) sum of rainfall for each week (dates indicated correspond to the fourth day of each week).

leaf (FL–1) was visually estimated when each cultivar reached the following growth stages: booting stage (GS 49), beginning of anthesis (GS 61) and early dough stage (GS 82). This was 20, 30 and 45 days after the second inoculation. In 1997, the evaluations were done on the same leaves at heading (GS 56), anthesis complete (GS 68) and soft dough stage (GS 84). This occurred at 27, 39 and 49 days after the second inoculation. Twenty plants were scored from the 2nd and 6th rows of each plot. Plant height, measured from the soil to the flag leaf and heading date, the time from planting to when 50% of the spikes emerged from the boot, were also evaluated in each plot. Area under disease progress curve (AUDPC) for each cultivar and each treatment was calculated to summarise the progress of the disease, according to the formula of Shaner and Finney (1977).

Data were analysed by means of a combined ANOVA for split plots across years and then for each year separately (Steel and Torrie, 1980). Heading date was used as a covariate. Means were compared with an LSD test.

Results

Temperatures and humidity were similar for both years although maximum temperatures were higher in 1996. Considering the whole period after inoculations (from inoculations till GS 84) minimum, average and maximum temperatures were 11.8, 16.3 and 22.1 °C for 1996 and 11.4, 16.0 and 20.6 °C for 1997, respectively (Fig. 5.1A). Minimum, average and maximum humidities for the same period were 53.7, 78.2 and 93.5% and 56.2, 78.5 and 95.6% for both years, respectively (Fig. 5.1B).

During September 1996, monthly precipitation was 102.8 mm, whereas in September 1997 there was no rainfall. During October, rainfall was scarce in both years (38.1 and 69.4 mm, respectively) but with a better distribution in 1996. There was no precipitation at the beginning of October 1997, immediately after inoculation. In November, the sum of rainfall was 84 and 133 mm, respectively, for both years (Fig. 5.1C).

The average percentages of necrosis of the two upper leaves (unadjusted means) on each evaluation date for each cultivar and fertilisation condition for both years are shown in Fig. 5.2. In 1996, increases of percentage of necrosis due to N fertilisation were significant at GS 61 (P=0.10) with averages of 10.89 and 5.49% for the fertilised and non-fertilised treatments, respectively, and at GS 82 (P=0.05) with 46.35 and 35.70% for the fertilised and non-fertilised treatments, respectively. Interactions cultivar × fertilisation were significant at GS 61. Significant increments for the N treatment were found for B. Ombú, D. Ernesto, P.I. Federal and P.I. Isla

Verde at GS 61, and for D. Ernesto, K. Dragón and P.I. Isla Verde at GS 82. In 1997, there were no significant differences between N fertilisation treatments at any growth stage. Interactions cultivar × N fertilisation were not significant either (Fig. 5.2). Differences between cultivars were significant for both years at dough stage (GS 82 or GS 84 for 1996 and 1997, respectively) and at GS 61 in 1996. The most resistant cultivars were K. Dragón, K. Centauro and P.I. Federal (data not shown).

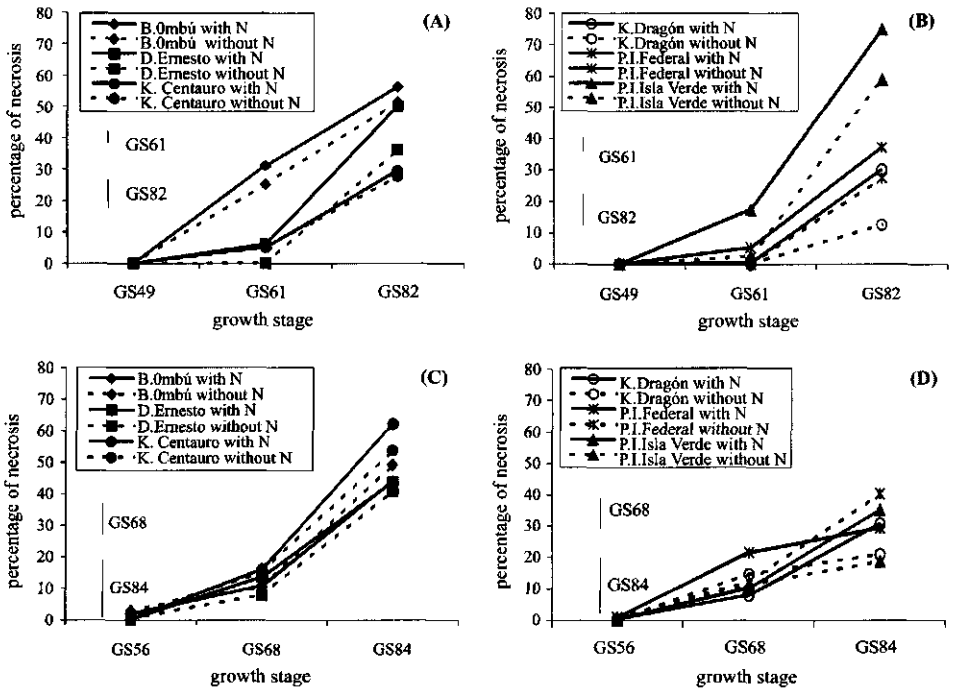

Fig. 5.2. Progress of septoria tritici blotch in six wheat cultivars over two years; (A) 1996, %necrosis for the cultivars Buck Ombú, Don Ernesto INTA and Klein Centauro with 100 kg ha^{-1} urea at sowing and without nitrogen; (B) 1996, %necrosis for the cultivars Klein Dragón, ProINTA Federal and ProINTA Isla Verde with 100 kg ha^{-1} urea at sowing and without N; (C) 1997, %necrosis for the cultivars Buck Ombú, Don Ernesto INTA and Klein Centauro with 100 kg ha^{-1} urea at sowing + 50 kg ha^{-1} at GS 30 and without nitrogen; (D) 1997, %necrosis for the cultivars Klein Dragón, ProINTA Federal and ProINTA Isla Verde with 100 kg ha^{-1} urea at sowing + 50 kg ha^{-1} at GS30 and without nitrogen. Bars indicate LSD (P=0.05) for comparisons of nitrogen treatments within each wheat cultivar.

Significant increments for the N treatment were found for B. Ombú, D. Ernesto, P.I. Federal and P.I. Isla Verde at GS 61, and for D. Ernesto, K. Dragón and P.I. Isla Verde at GS 82. In 1997, there were no significant differences between N fertilisation treatments at any growth stage. Interactions cultivar × N fertilisation were not significant either (Fig. 5.2). Differences between cultivars were significant for both years at dough stage (GS 82 or GS 84 for 1996 and 1997, resp.) and at GS 61 in 1996.

AUDPC was not associated with heading date or plant height when analysed over both years. However, AUDPC was negatively associated with heading date in 1996 (r=–0.62*) and positively associated in 1997 (r=0.70**). For that reason it was included as a covariate in the analysis of variance. Averages for heading date for each year, cultivar, and N-fertilisation treatment are indicated in Table 5.1. Comparisons of heading date indicated a reduction in the days to heading in 1997 compared to 1996, mainly because of a later sowing. K. Centauro was the latest-maturing cultivar in both years. The earliest-maturing cultivars were B. Ombú in 1996 and P.I. I. Verde in 1997. This indicates that P.I. I. Verde was more affected by the late sowing in 1997, probably due to a high sensitivity to photoperiod.

Years, cultivars, N treatments and year × cultivar and year × N interactions were significant (Table 5.2) for the AUDPC values. Due to significant interactions a separate ANOVA was performed for each year (Table 5.3).

In 1996, significant differences in the AUDPC between N treatments were observed (Table 5.3). The interaction N fertilisation × cultivar was also significant. In

Table 5.1. Days to heading for six wheat cultivars under two nitrogen fertilisation treatments in two years.

Cultivar	Days to heading					
	1996			1997		
	With fertiliser	Without fertiliser	Averages	With fertiliser	Without fertiliser	Averages
Buck Ombú	91.7 a[y]	91.7 a	91.7 A[z]	91.0 a	91.0 a	91.0 B
Don Ernesto	94.0 a	93.0 b	93.5 C	91.0 a	91.0 a	91.0 B
Klein Centauro	102.0 a	102.0 a	102.0 E	94.0 a	94.0 a	94.0 C
Klein Dragón	96.5 a	94.0 b	95.2 D	91.7 a	91.0 a	91.3 B
ProINTA Federal	94.0 a	93.0 b	93.5C	91.0 a	91.0 a	91.0 B
ProINTA I. Verde	93.0 a	92.5 a	92.7 B	85.0 a	85.0 a	85.0 A
Averages	95.2 a	94.4 b		90.6 a	90.5 a	

[y] Means followed by the same letter in the same row within the same year are not significantly different, LSD (P=0.05).

[z] Means followed by the same letter in the average columns within the same year are not significantly different, LSD (P=0.05).

1997, differences between N treatments or the interaction N fertilisation × cultivar were not significant. Differences between cultivars were significant in both years.

Adjusted (by the covariate) and non-adjusted means are presented to evaluate the influence of the heading date in the AUDPC values (Table 5.4). Although evaluations were performed some days earlier in 1996 than in 1997, the average of the AUDPC in 1996 was higher compared to 1997 due to higher disease severities caused by differences in environmental conditions. In 1996, the average of the AUDPC for the six cultivars was higher in the N treatment than in the control. Considering each cultivar, D. Ernesto, K. Dragón, P.I. Federal and P.I. I. Verde evidenced significantly higher values in the fertilised treatments than in the non-fertilised ones. Cultivars had in general differences in their resistance level with K. Dragón being the most resistant in both years.

To find an explanation for the differences between years for N treatments, the AUDPC of the two upper leaves were analysed separately in each year. Heading date was included as a covariate because it was negatively associated with the AUDPC of the two upper leaves in 1996 ($r=-0.70^{***}$ and $r=-0.55^{*}$ for the FL and FL–1 respectively) and positively associated with the AUDPC of the two upper leaves in 1997 ($r=0.69^{***}$; $r=0.66^{**}$ for the FL and FL–1, respectively). In 1996, there were differences between fertilisation treatments for the two leaves analysed. D. Ernesto, P.I. Federal and P.I. Isla Verde showed higher AUDPC in the FL in the N treatment and D. Ernesto, K. Dragón and P.I. Isla Verde in the FL–1. In 1997, there were significant differences between N treatments for the FL but not for the FL–1. In the FL, K. Centauro showed the most important differences, followed by D. Ernesto.

Table 5.2. Combined analysis of variance for the AUDPC of septoria tritici blotch on six wheat cultivars under two nitrogen fertilisation treatments in two years.

Source of variation	df	Mean squares	P>F [x]
Year (Y)	1	62478	0.0471
Error a	3	5877	
N-Fertilisation (N)	1	209629	0.0014
Y x N	1	64201	0.0178
Error b	6	6131	
Cultivar(C)	5	207226	<0.0001
Y x C	5	242392	<0.0001
N x C	5	13960	0.0756
Y x N x C	5	5880	0.4937
Covariant: heading	1	507	0.7854
Error c	59	6588	

[x] Probability level, F-test.

Table 5.3. Separate analysis of variance for the AUDPC of septoria tritici blotch on six wheat cultivars under two nitrogen fertilisation treatments in two years.

Source of variation	df	Mean squares			
		1996		1997	
N-Fertilisation (N)	1	223361	(0.0175)[z]	7213	(0.2371)
Error a	3	9811		3321	
Cultivar (C)	5	315365	(<0.0001)	24784	(0.0216)
N × C	5	14232	(0.0350)	5201	(0.6565)
Covariant: heading	1	398400	(<0.0001)	146729	(0.0002)
Error b	29	5080		7878	

[z] P>F

Table 5.4. Means of the AUDPC of septoria tritici blotch on six wheat cultivars under two nitrogen fertilisation treatments in two years.

Cultivar	AUDPC					
	1996			1997		
	With fertiliser	Without fertiliser	Average	With fertiliser	Without fertiliser	Average
Buck Ombú	723 a[y] (812)[x]	634 a (702)	679[z] E (757)	343 a (351)	370 a (380)	356 B (365)
Don Ernesto	428 a (459)	237 b (273)	332 B (366)	362 a (370)	286 a (295)	324 AB (333)
Klein Centauro	505 a (330)	466 a (272)	486 C (301)	420 a (489)	374 a (445)	397 B (467)
Klein Dragón	265 a (231)	85.3 b (96)	175 A (163)	222 a (246)	258 a (268)	240 A (257)
ProINTA Federal	313 a (343)	169 b (205)	241 A (274)	382 a (391)	332 a (342)	357 B (366)
ProINTA Verde	721 a (778)	423 b (472)	572 D (625)	406 a (292)	336 a (225)	371 B (258)
Averages	492 a	336 b		357 a	326 a	

Means are adjusted by heading date as a covariant.

[x] Unadjusted values. [y] Means followed by the same letter in the same row within the same year are not significantly different, LSD (P=0.05). [z] Means followed by the same letter in the average columns within the same year are not significantly different, LSD (P=0.05).

Differences between cultivars were significant for all leaves, except the FL–1 in 1997. Klein Dragón and P.I. Federal showed the lowest AUDPC values in both leaves in 1996 and in the FL in 1997. In spite of the non-significant differences between cultivars in the ANOVA for the FL–1 in 1997, K. Dragón showed higher levels of resistance than the other cultivars according to the LSD test (P=0.05) (comparisons not shown). The interaction cultivar × N-fertilisation was significant for both leaves only in 1997 (Tables 5.5 and 5.6).

Discussion

The amount of N applied in these experiments can be considered high for the wheat growing area of Argentina, although they are moderate for intensive growing conditions. The aim was to study the effect of these fairly high rates on the progress of the disease and in future studies to use several rates to evaluate the response to N as a quantitative variable. However, the increment of the severity of septoria tritici blotch using these high rates for our extensive growing conditions, although significant, was not high. Differences between fertilisation treatments were higher comparing the AUDPC values than comparing percentages of necrosis at each growth stage, due to the cumulative effects of increments at each particular growth stage, especially in 1996. Differences for necrosis percentage between N treatments were more significant at advanced growth stages because at GS 49 or GS 56 the disease was just starting.

Weather was more conducive to the disease in 1996 than in 1997 because of the higher amount of precipitation after inoculations. In both years, average temperatures were near the optimum range for the development of the septoria tritici blotch. Under greenhouse conditions, temperatures from 17 to 25 °C are optimum for disease development (Hess and Shaner, 1987; Shaw, 1990; Wainshilbaum and Lipps, 1991). Some interactions between temperature and cultivars were also found, with higher AUDPC values at 19 °C in some cultivars (Wainshilbaum and Lipps, 1991).

The heading date of the cultivars and its relation with weather influenced the expression of the resistance creating a more favourable environment for a specific type of cultivar (late or early heading). Genetic resistance is unrelated to maturity (Arama et al., 1999; Simón et al., 2001, 2002). In 1996, heading date was negatively associated with the AUDPC. Although this agrees with previous reports (Tavella, 1978; Van

Table 5.5. Analysis of variance for the AUDPC of septoria tritici blotch on each of the two upper leaves of six wheat cultivars under two nitrogen fertilisation treatments in two years.

Source of variation	df	Mean squares			
		1996		1997	
		Flag leaf	Flag leaf-1	Flag leaf	Flag leaf-1
N Fertilisation (N)	1	29303 (0.0131)[y]	599138 (0.0305)	10894 (0.0178)	4288 (0.5687)
Error a	3	1041	39948	484	10165
Cultivar (C)	5	88186 (<0.0001)	816980 (<0.000)	9265 (0.0018)	56889 (0.0521)
N x C	5	10896 (0.1410)	22460 (0.1115)	6354 (0.0135)	123777 (0.0011)
Covariant: heading	1	250875 (<0.0001)	579890 (<0.0001)	44706 (<0.0001)	307656 (0.0009)
Error b	29	6001	11314	1815	22615

[y] P>F

Table 5.6. Means of the AUDPC of septoria tritici blotch on the two upper leaves of six wheat cultivars under two nitrogen fertilisation treatments in two years.

	Flag leaf		Flag leaf-1		Flag leaf		Flag leaf-1	
Cultivar	With fertiliser	Without fertiliser	With fertiliser	Without fertiliser	With fertiliser	Without fertiliser	With fertiliser	Without fertiliser
Buck Ombú	226 a (296)[z]	259 a (313)	1220 a (1327)	1008 a (1090)	79.2 a (83.7)	139 a (145)	606 a (618)	601 a (615)
Don Ernesto	280 a (304)	172 b (200)	576 a 613)	302 b (345)	124 a (128)	45.3 b (50.9)	600 a (611)	526 a (540)
Klein Centauro	213 a (74.3)	234 a (79.4)	797 a (586)	699 a (464)	194 a (232)	93.0 b (132)	647 a (746)	656 a (758)
Klein Dragón	117 a (90.8)	58.0 a (66.2)	412 a (372)	113 b (125)	74.5 a (87.4)	39.4 a (45.0)	370 a (404)	477 a (492)
ProINTA Federal	156 a (181)	67.2 b (96.2)	469 a (506)	271 a (314)	83.0 a (87.5)	68.3 a (73.8)	682 a (694)	595 a (609)
ProINTA Isla Verde	456 a (501)	287 b (325)	987 a (1055)	559 b (618)	144 a (81.2)	105 a (44.0)	669 a (505)	567 a (406)
Averages	241 a	180 b	743 a	492 b	116 a	81.7 b	596 a	570 a

[z]Means are adjusted by heading date as a covariant. [z]Unadjusted values.
Means followed by the same letter within the same row for each leaf within the same year are not significantly different, LSD (P=0.05).

Beuningen and Kohli, 1990) it is necessary to consider that probably different environmental conditions can modify this tendency. Early cultivars were exposed to lower maximum temperatures after FL emergence compared to late cultivars. During this year the FL emerged between 8 and 20 October for early and late cultivars and maximum temperatures were unfavourable (more than 27 °C) for the development of septoria tritici blotch after the first week of November. Progress of the disease in the FL–1 could also be delayed in late cultivars due to these environmental conditions. The earliest cultivar, B. Ombú, was the most susceptible. This was partially due to its earliness as it is shown in the adjusted means when data are corrected using heading date as a covariate.

In 1997, heading date was positively associated with the AUDPC. This can be explained by the increase in the AUDPC of the late heading cultivar (K. Centauro) probably due to the increase in rainfall from the appearance of its flag leaf (FL emerged between 22 and 31 October for early and late cultivars). At this time FL–1 was still young, thus the percentage of necrosis also increased, although differences between fertilisation treatments were only significant for the FL which was exposed to a higher amount of precipitation from the beginning. Moisture in the form of free water or water vapour is important in the epidemiology of septoria tritici blotch (King et al., 1983). On the other hand, the earliest cultivar in this year (P.I. Isla Verde) showed a lower AUDPC (unadjusted mean) that could be caused by the lower severity induced by the dry condition after the flag leaf appearance. In addition, during this growing season maximum temperatures were lower than in 1996.

Differences in rainfall immediately after inoculation in both years can explain why only in 1996 the average of the AUDPC was higher in the treatments with additional N-fertiliser than in the unfertilised control. Spore germination is enhanced by longer periods of leaf wetness (Holmes and Cohloun, 1974), so a denser canopy resulting from additional N (Seligman et al., 1983) should increase spore germination. The scarce precipitation in September and the beginning of October 1997 could have reduced spore germination in such a way that differences in canopy were not enough to increase the average of AUDPC in the fertilised treatment.

A deeper study of the canopy structure of the cultivars should be done. However in some cultivars, P.I. Federal and P.I. I. Verde, with higher necrosis values in the N-fertilised treatment in 1996 than in the non-fertilised one, shorter distances between their upper leaves with respect to the other cultivars were observed (data not shown).

The increase in the septoria tritici blotch severity under N-fertilisation agreed with reports by other authors (Gheorghies, 1974; Prew et al., 1983; Broscious et al., 1985; Hayden et al., 1994; Howard et al., 1994; Leitch and Jenkins, 1995). Some other reports are contradictory to these results. This may be due to several factors, such as

type and level of fertility of the soil, rate and type of fertiliser applied, weather conditions, crop husbandry (tillage or non-tillage practices, use of fungicides), etc. which could have been influencing the effect of the fertilisation on the disease expression.

Arama (1996) found significant differences between N treatments in soils with a good level of N and without irrigation but not in poor soils under irrigation. He argued that this was probably because the amount of N applied was not large enough for the poor soil and because N fertiliser could have been leached under irrigation. Tompkins et al. (1993) found an increase in septoria tritici blotch severity with low N fertilisation in one of his experiments, but that study was conducted in a no-till seeding wheat, where plants without fertilisation were weaker than in conventional tillage and more susceptible to the disease. Hayden et al. (1994) did not find differences between N-fertilisation treatments, but the source of N was a previous one-season legume crop that, as the authors speculated was not probably enough to create differences.

Considering amounts and types of N applied in our and other experiments a range from 65 kg to 300 kg N ha^{-1} (Prew et al., 1983; Leitch and Jenkins, 1995; Arama et al., 1996) increased the severity of the disease under conducive environmental conditions. Arama (1996) and our experiments showed that calcium ammonium nitrate and urea, respectively, increased the disease in conducive conditions. With regard to the time of application in our experiments and in several other reports, increments in disease severity were observed when fertiliser was applied from sowing to GS 38 (Leitch and Jenkins, 1995; Arama et al., 1996).

We can conclude that N fertilisation causes increases in septoria tritici blotch under conducive weather conditions. Discrepancies among reports could be partially due to the fact that weather conditions were not conducive to the development of the disease or soil conditions were not suitable for the best utilisation of the N applied. Selection of breeding materials with moderate levels of fertiliser-N could contribute to obtain resistant materials for those conditions. Farmers should consider the influence of N applications in the expression of septoria tritici blotch and its possible consequences in yield reduction.

CHAPTER 6

Influence of septoria tritici blotch (*Mycosphaerella graminicola*) on yield, yield components and test weight of wheat under two nitrogen fertilisation conditions[1]

[1] This chapter has been published as:
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Materials and methods

Two experiments were conducted at the Estación Experimental J. Hirschhorn, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, during 1996 and 1997. The trials were sown on 17 July 1996 and 5 August 1997 under conventional tillage. The soil was a Typic Argiudol. Analysis of the soil samples (top-0.20m) indicated the following values by weight: organic matter = 3%; N = 0.17%; P = 7 mg kg^{-1}, and pH = 5.9. Average temperatures and relative humidity for the entire period after inoculations were 16.3 °C and 78.2% for 1996 and 16.0 °C and 78.5% for 1997. Precipitations after inoculations were 103 mm and 0 mm in September; 38 mm and 69 mm in October and 84 mm and 133 mm in November for 1996 and 1997, respectively.

The experimental design was a split-split-plot with four replications. Main plots were inoculated and non-inoculated treatments. Subplots were fertilisation treatments, 0 and 100 kg N ha^{-1} as urea at sowing in 1996, and 0 and 100 kg N ha^{-1} of urea at sowing plus 50 kg N ha^{-1} as urea at Growth Stage (GS) 30 (Zadoks et al., 1974) in 1997. Cultivars Buck Ombú (B. Ombú), Don Ernesto INTA (D. Ernesto), Klein Centauro (K. Centauro), Klein Dragón (K. Dragón), ProINTA Federal (P.I. Federal), and ProINTA Isla Verde (P.I. I. Verde) were the sub-subplots. These cultivars were known to differ in their resistance to the disease according to the information provided by their respective breeders. Between the main plots, three plots of oat were sown to avoid the spread of inoculum between inoculated and non-inoculated treatments. The same was done between blocks. Plots were 6.3 m^2 (4.50 m long × 1.4 m wide). The entire experiment was fertilised with 50 kg P_2O_5 ha^{-1} as calcium triple superphosphate at the time of sowing.

A virulent isolate (FALP 3096, Facultad de Ciencias Agrarias y Forestales de La Plata, Argentina) of *M. graminicola* was used to prepare the inoculum. The isolate was grown on malt extract agar at 19 °C with 12 h alternating light and dark cycles. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in de-ionised water. The conidial suspension was adjusted to 5×10^6 spores mL^{-1} and 0.5 ml of Tween 20 per litre was added as a surfactant. Two inoculations were done (at the beginning of tillering, GS 21, and at the beginning of shoot development, GS 31, in both years). Plants were sprayed with the inoculum suspension until runoff. After inoculations, plants were kept moist by spraying with water several times a day with sprinklers, during a period of 3 days. Inoculated plots were sprayed with Plantvax (oxicarboxin; 5,6 dihydro-2 methyl-N-phenyl-1,4-oxathiin-3-carboxamide 4,4-dioxide; Dhanuka Group, New Dehli, India) several times during the growing season to prevent rust infection. The non-inoculated treatment (control) was sprayed with Plantvax and Folicur {tebuconazole; alpha-[2-(4-

chlorophenyl) ethyl] alpha-(1,1-dimethylethyl)-1H-1,2,4-triazol-1 ethanol; Bayer Corp., Leverkusen, Germany} to prevent rusts and septoria leaf blotch.

Disease evaluations were done on the upper two leaves of 20 plants from the second and sixth rows when each cultivar reached GS 49, GS 61 and GS 82 in 1996, and GS 56, GS 68 and GS 84 in 1997. Yield components (EPM^2, KPE, and TKW) and TW were evaluated in each plot. Three 1-m long sections from the three central rows in each plot were harvested at random and the number of ears were counted to determine EPM^2. From that sample, KPE were determined on 30 spikes, threshed and the grains counted by means of a mechanical counter. The grain counted in the 30 ears were weighed to determine TKW (g). Test weight was determined with a Schopper scale, which weighs a volume of 250 cm^3 and converts it to 1 hl by means of a table. Four meters of the three central rows (including the sections harvested for EPM^2) in each plot were harvested and threshed (area 2.4 m^2) and the grain yield (kg ha^{-1}) was calculated.

Data were analysed by ANOVA for split-split plot designs in a combined analysis for both years. Because of some significant year interactions a separate analysis for each year was also performed. Percentages of reductions due to *M. graminicola* for yield, yield components, and TW in both fertilisation levels relative to the non-inoculated control were also calculated. These results were analysed by ANOVA for split-plots, with fertilisation as main plot factors and cultivars as subplot factors. Least significant differences (LSD) were calculated for mean separation.

The area under disease progress curve (AUDPC) values of septoria tritici blotch measured in the two upper leaves were calculated according to the formula of Shaner and Finney (1977). Those values were correlated with the percentage of yield reduction relative to the non-inoculated control in each cultivar by means of a regression line.

Results

Analysis of variance and averages of AUDPC values of septoria tritici blotch in the upper two leaves of the inoculated treatment for both years and both fertilisation treatments are indicated in Tables 6.1 and 6.2, respectively. There were significant differences for fertilisation, cultivars and the interaction fertilisation × cultivar in 1996 and for cultivars only in 1997.

The combined analysis for yield and yield components for both years (Table 6.3) showed that year was significant for TKW and TW. Inoculation and cultivar effects were significant for all traits. Differences between N-treatments occurred for

yield, EPM^2 and KPE. Interaction year × inoculation was not significant for any trait. Year × fertilisation effects were significant for yield, EPM^2 and year × cultivar for all traits. Inoculation × fertilisation, inoculation × cultivar, and fertilisation × cultivar interactions were not significant for any trait. There were no significant triple or quadruple interaction effects. Because of the significant year effects and the interactions with year, a separate analysis was performed for 1996 and 1997.

For the separate analysis, Table 6.4 shows that the inoculation with *M. graminicola* reduced the yield and KPE in both years. Ears per square meter, TW and TKW were reduced significantly only in 1996. N-fertilisation increased yield, EPM^2 and KPE in both years. Thousand-kernel weight and TW were not modified by fertilisation. Differences between cultivars were significant for all traits except for EPM^2 in

Table 6.1. Mean squares of the area under disease progress curve values for septoria tritici blotch on six wheat cultivars under two N-fertilisation treatments in two years.

Source of variation	df	1996	1997
Fertilisation (F)	1	291101^{*}	11124^{ns}
Error a	3	10109	3943
Cultivar (C)	5	414980^{***}	49716^{**}
Interaction F × C	5	14321^{*}	4056^{ns}
Error b	15	4911	8412

* significant at the 0.05 probability level; ** significant at the 0.01 probability level; *** significant at the 0.001 probability level; ns, not significant.

Table 6.2. Means of the area under disease progress curve (two upper leaves) of septoria tritici blotch on six wheat cultivars under two N-fertilisation conditions in two years.

Cultivar	1996			1997		
	With fertiliser	Without fertiliser	Average	With fertiliser	Without fertiliser	Average
Buck Ombú	812	702	757	351	380	365
Don Ernesto	459	273	366	370	296	333
Klein Centauro	330	272	301	489	445	467
Klein Dragón	231	96	163	246	268	257
ProInta Federal	343	205	274	391	342	366
ProInta Isla Verde	778	472	625	292	225	258
Average fertilisation	492	337	414	356	326	341
LSD fertilisation	92.5			57.7		
LSD cultivars	74.9			93.6		

1996, and KPE in both years. Differences between cultivars were significant for all traits except for EPM^2 in 1996. Two-way interactions were not significant, except for inoculation × cultivar for TKW in 1996. Three-way interaction was not significant either.

On average, reduction in yield due to the inoculation was 27.7% in 1996 and 26.0% in 1997. Mean increases in yield due to fertilisation were 23.5% in 1996 and 12.6% in 1997. When the reductions in yield due to inoculation were compared between the fertilisation treatments, the percentages were similar (29.7% and 25.7% in 1996, and 26.6 and 25.4% in 1997 for the non-fertilised and the fertilised treatment, respectively). Differences in yield reduction due to septoria tritici blotch between both fertilisation conditions within each year were not significant (Table 6.5, Fig. 6.1).

Cultivars differed in their resistance levels in both years (Table 6.1). However differences in yield reduction due to septoria tritici blotch (Table 6.5, Fig. 6.2) were significant only in 1996 when differences in AUDPC values were greater. In 1997,

Table 6.3. Mean squares form the combined analysis of two years for yield, yield components and test weight for six wheat cultivars under two N-fertilisation conditions and two inoculation treatments with *M. graminicola*.

Source of variation	df	Yield ha^{-1}	Ears m^{-2}	Kernels ear^{-1}	1000-Kernel weight	Test weight
Main plots						
Year (Y)	1	45538000ns§	35734ns	227.1ns	690.6***	717.6**
Error a	3	14176297	16496	69.9	14.5	11.9
Subplots						
Inoculation (I)	1	211400000***	100278***	1142***	388.6***	73.4**
Y × I	1	2348803ns	8199ns	7.47ns	11.0ns	0.15ns
Error b	6	727626	3727	21.1	10.5	3.87
Sub-subplots						
Fertilisation (F)		62113000***	184148***	314.6**	1.88ns	11.5ns
Y × F	1	7315784*	318010***	4.59ns	7.64ns	3.77ns
I × F	1	162753ns	7904ns	2.23ns	10.7ns	5.27ns
Y × I × F	1	1710ns	1039ns	7.34ns	4.56ns	0.92ns
Error c	12	1266184	3196	24.8	3.29	9.58
Sub-sub-subplots						
Cultivator (C)	5	16947000***	36369***	677.4***	251.9***	126.8***
Y × C	5	5821862**	45630***	50.9**	19.3*	30.4***
I × C	5	1526192ns	4983ns	2.34ns	14.8ns	7.97ns
F × C	5	1115661ns	2258ns	25.6ns	2.81ns	5.84ns
Y × F × C	5	1473485ns	1195ns	3.21ns	3.49ns	5.40ns
Y × I × C	5	595464ns	1390ns	18.9ns	9.59ns	3.75ns
I × F × C	5	45492ns	11978ns	7.55ns	3.99ns	1.64ns
Y × I × F × C	5	75815ns	6704ns	3.75ns	1.23ns	4.80ns
Error d	120	1811392	6366	17.3	6.83	3.94

* Significant at the 0.05 probability level;** significant at the 0.01 probability level;
*** significant at 0.001 probability level; §ns, not significant.

Table 6.4. Mean squares for the separated analysis of two years for yield, yield components and test weight for six wheat cultivars under two N fertilisation conditions and two inoculation treatments with *Mycosphaerella graminicola*.

Source of variation	df	Yield ha^{-1}		Ears m^{-2}		Kernels ear^{-1}		1000 Kernel weight		Test weight	
		1996	1997	1996	1997	1996	1997	1996	1997	1996	1997
Main plot											
Inoculation (I)	1	129160000*	84592565***	82912**	25565ns§	482.5*	667.3**	265.1***	134.5ns	33.4**	40.1ns
Error a	3	1232400	222853	1071	6384	28.5	13.6	1.32	19.8	0.85	6.89
Subplots											
Fertilisation (F)	1	56031000***	13397640*	184515***	31443*	197.6*	121.6*	8.54ns	0.97ns	14.2ns	1.05ns
I x F	1	65551ns	98912ns	7337ns	1606ns	0.74ns	8.83ns	2.77ns	1.84ns	0.89ns	5.30ns
Error b	6	109654	1436213	2413	3980	32.63	17.1	2.94	3.63	15.3	3.89
Sub-subplots											
Cultivar (C)	5	12241000***	10527272***	716.6ns	81282***	234.9***	493.3***	138.7***	132.5***	64.1***	93.1***
I x C	5	1992354ns	129301ns	4076ns	2297ns	16.3ns	15.8ns	20.4***	4.01ns	11.1ns	0.67ns
F x C	5	1765793ns	823353ns	3526ns	1549ns	3.87ns	1.69ns	2.75ns	3.55ns	4.48ns	5.7ns
I x F x C	5	110542ns	10766ns	1313ns	1473ns	3.10ns	8.21ns	3.56ns	1.66ns	2.75ns	3.69ns
Error c	60	1978080	1644705	5395	7338	13.0	21.6	3.18	10.5	5.43	2.45

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at 0.001 probability level
§ns, not significant

Table 6.5. Means of yield for six wheat cultivars under two N fertilisation conditions and two inoculation treatments with *Mycosphaerella graminicola*.

	1996				1997					
	With fertiliser		Without fertiliser		With fertiliser		Without fertiliser			
Cultivar	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	Average 1996	Average 1997
	kg ha^{-1}									
Buck Ombú	5305(38.1)+	8579	4501(44.9)	8166	5097(31.3)	7423	4822(30.6)	6951	6638	6074
Don Ernesto	6521(26.3)	8852	4929(32.0)	7251	5157(27.0)	7062	4176(29.5)	5925	6888	5580
Klein Centauro	6835(18.6)	8400	5836(20.8)	7371	7413(19.5)	9213	5961(22.0)	7644	7111	7558
Klein Dragón	9325(16.6)	11175	6974(17.7)	8474	6512(20.8)	8223	5798(23.5)	7580	8987	7029
ProINTA Federal	6524(25.3)	8744	4713(31.6)	6888	5035(28.2)	7015	4760(27.0)	6525	6717	5834
ProINTA Isla Verde	6550(31.4)	9542	5252(31.5)	7661	4950(28.0)	6879	4550(28.0)	6321	7251	5675
Average cultivars	6843(25.7)	9215	5367(29.7)	7635	5694(25.4)	7636	5011(26.6)	6824	7265	6291
Average fertilisation										
With	8029				6665					
Without	6501				5918					
Average inoculation										
With	6105				5353					
Without	8425				7230					
LSD cultivars	249.0				906.8					
LSD fertilisation	522.9				598.6					
LSD inoculation	721.0				306.6					

+ Percentage of reduction relative to the non -inoculated control are given in parenthesis.

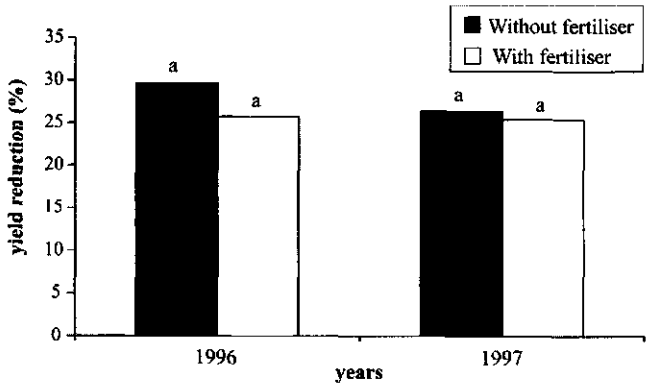

Fig. 6.1. Average percentage of yield reduction of six wheat cultivars inoculated with *M. graminicola* in two fertilisation conditions and two years. Means followed by the same letter within the same year are not statistically significant at P<0.05.

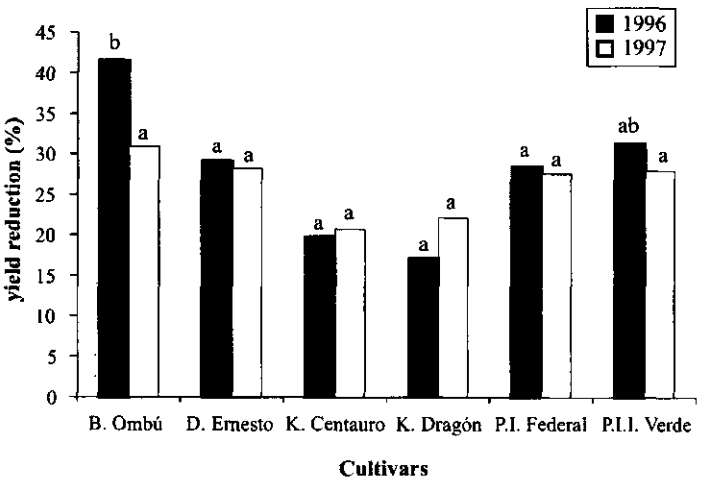

Fig. 6.2. Percentages of yield reduction in six wheat cultivars inoculated with *M. graminicola* in two years. Means followed by the same letter within the same year are not significantly different. B. Ombú, 'Buck Ombú'; D. Ernesto 'Don Ernesto'; K. Centauro, 'Klein Centauro; K. Dragón 'Klein Dragón'; P.I. Federal, 'ProINTA Federal'; P.I.I. Verde, 'ProINTA Isla Verde'.

reductions in yield tended to be similar among the cultivars. Cultivars K. Centauro and K. Dragón had high yields in both years and had a lower reduction in yield due to the inoculation compared to B. Ombú in 1996 (Fig. 6.2).

Neither the fertilisation main effect for the reduction nor the cultivar × fertilisation interaction was significant for any of the yield components or TW (ANOVA not shown). The reduction in EPM^2 due to the inoculation was 7.0 and 14.6% and 4.0 and 7.3% in 1997 for the fertilised and non-fertilised treatment, respectively (Table 6.6). Kernels per ear was reduced in inoculated plots by 10.9 and 12.8% in 1996 and 15.6

Table 6.6. Means of ears per meter for six wheat cultivars under two N fertilisation conditions and two inoculation treatments with *M. graminicola*.

	1996				1997					
	With fertilisation		Without fertilisation		With fertilisation		Without fertilisation			
Cultivar	With inoculation	Without inoculation	With Inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	Average 1996	Average 1997
	Ears m^{-2}									
Buck Ombú	540(10.0)§	600	466(14.6)	546	512(6.70)	549	497(3.10)	513	538	518
Don Ernesto	539(11.5)	609	443(19.3)	549	600(5.10)	632	511(13.1)	588	535	583
Klein Centauro	550(5.80)	584	469(5.40)	496	698(1.96)	712	651(8.18)	709	525	693
Klein Dragón	609(0.16)	610	471(3.50)	488	560(2.75)	545	490(5.77)	520	545	529
ProINTA Federal	580(3.60)	602	415(22.6)	536	565(1.07)	559	540(3.57)	560	533	556
ProINTA Isla Verde	527(10.4)	588	450(19.2)	557	470(15.5)	556	450(9.05)	495	530	493
Average cultivars	557(7.00)	599	452(14.6)	529	568(4.05)	592	523(7.30)	564	534	562
Average fertilisers										
With	578				579					
Without	491				544					
Average inoculation										
With	505				545					
Without	564				580					
LSD cultivars	51.9				60.6					
LSD fertilisers	15.7				31.5					
LSD inoculation	21.3				51.9					

§ Percentages of reduction relative to the non inoculated control are given in parenthesis

Table 6.7. Means of kernels per ear for six wheat cultivars under two N fertilisation conditions and two inoculation treatments with *M. graminicola*

	1996				1997					
	With fertilisation		Without fertilisation		With fertilisation		Without fertilisation			
	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	Average 1996	Average 1997
	Kernels ear^{-1}									
Buck Ombú	30.0 (20.8)§	37.9	29.5(18.3)	36.1	31.0(14.4)	36.2	29.4(12.2)	33.5	33.4	32.5
Don Ernesto	34.0(5.82)	36.1	31.5(4.55)	33.0	26.8(14.1)	31.2	24.8(15.9)	29.5	33.7	28.2
Klein Centauro	33.6(14.7)	39.4	29.2(18.9)	36.0	27.1(22.6)	35.0	27.3(10.5)	30.5	34.5	30.0
Klein Dragón	41.7(7.95)	45.3	38.8(5.83)	41.2	37.9(12.5)	43.3	37.6(6.70)	40.3	41.7	39.7
ProINTA Federal	32.5(8.45)	35.5	27.5(18.6)	33.8	29.2(14.1)	34.0	26.9(11.2)	30.3	32.3	30.1
ProINTA Isla Verde	39.0(8.45)	42.6	36.1(10.9)	40.5	39.1(15.4)	46.2	35.4(22.0)	45.4	39.5	41.5
Average cultivars	35.1(10.9)	39.4	32.1(12.8)	36.8	31.9(15.6)	37.8	30.2(13.5)	34.9	35.9	33.7
Average fertilisation										
With	37.3				34.8					
Without	34.4				32.6					
Average inoculation	34.4				32.6					
With	33.6				31.1					
Without	38.4				36.3					
LSD cultivars	2.55				3.28					
LSD fertilisation	2.85				2.06					
LSD inoculation	3.47				2.40					

§Percentages of reduction relative to the non inoculated control are given in parenthesis.

and 13.5% in 1997 for the fertilised and non-fertilised treatment, respectively (Table 6.7). This increase in KPE reduction in 1997 can be attributed to the decrease in the reduction of EPM^2 compared with 1996. As it is known, yield components are not independent of each other. Thousand-kernel weight was reduced by 9.5 and 7.7% in 1996, and by 6.1 and 7.8% in 1997 (Table 6.8), and TW was reduced by 1.4 and 1.9%, in 1996 and by 1.2 and 2.3% in 1997 (Table 6.9) for the fertilised and non-fertilised treatments, respectively. There were significant differences between cultivars in reductions with respect to the control for TKW in 1996: K. Centauro and K. Dragón had the lowest reductions (ANOVA not shown).

The regression line between the AUDPC values (on the upper two leaves) and the reduction in yield due to *M. graminicola* was analysed for both fertilisation conditions and both years. In 1996, with conducive conditions for septoria tritici blotch, there was a good discrimination between susceptible and moderately resistant cultivars. Larger reductions in yield (Table 6.5) occurred on cultivars with a higher AUDPC (Table 6.2) in both fertilisation treatments, and the regression coefficients were significant (Fig. 6.3). In 1997, with unconducive environmental conditions, differences in AUDPC between cultivars were lower than in 1996, and the regression between the AUDPC and the yield reduction was not significant. In both years, but especially for 1997, K. Centauro and K. Dragón had lower reduction in yield than expected from the recorded disease severity on these cultivars.

Discussion

Inoculations done at early growth stages (GS 21 and GS 31) initiated disease during tillering. The reduction in EPM^2 in 1996 due to septoria tritici blotch could be caused by a decrease in the number of produced tillers but also by a reduction in their survival. In 1997, there was no significant reduction in the EPM^2. Scarce rain after inoculation could have caused a delay in the progress of the epidemic. Considering the upper two leaves, the severity of septoria tritici blotch was higher in 1996 (with conducive conditions) than in 1997.

Yield and the three yield components were reduced by inoculation with *M. graminicola*. Yield reductions fluctuated between 16.6 and 44.9% depending on the cultivar and fertilisation treatment. Values fell within the broad range quoted by other researchers (Eyal et al., 1987; Annone et al., 1991; Kraan and Nisi, 1993). Under natural infections caused by septoriosis and rusts, Leitch and Jenkins (1995) found significant reductions in yield, EPM^2 and kernel weight. Reductions were also found for KPE in high-fertilisation conditions. Analysing yield and yield component losses

Table 6.8. Means of 1000 kernel weight (g) for six wheat cultivars under two N fertilisation conditions and two inoculation treatments with *Mycosphaerella graminicola*.

	1996				1997					
	With fertilisation		Without fertilisation		With fertilisation		Without fertilisation			
Cultivar	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	Average 1996	Average 1997
	g									
Buck Ombú	36.4(9.84)	40.3	37.2(11.5)	42.0	34.2(5.08)	36.0	31.7(13.3)	36.6	39.0	34.6
Don Ernesto	33.3(12.5)	38.1	34.2(9.73)	37.9	31.1(11.6)	35.1	30.8(10.3)	34.3	35.9	32.8
Klein Centauro	40.5(0.00)	40.5	42.3(2.42)	43.4	38.0(3.13)	39.2	36.8(4.50)	38.6	41.7	38.2
Klein Dragón	38.6(2.60)	39.6	37.6(5.90)	40.0	31.5(6.39)	33.6	31.4(7.60)	34.0	38.9	32.6
ProINTA Federal	32.3(11.51)	36.5	33.7(9.13)	37.1	31.7(4.23)	33.1	33.1(4.96)	34.9	34.9	33.2
ProINTA Isla Verde	29.6(21.6)	37.8	31.3(11.6)	35.4	28.7(5.96)	30.5	28.4(5.06)	29.9	33.5	29.4
Average cultivars	35.1(9.54)	38.8	36.1(7.70)	39.1	32.5(6.06)	34.6	32.0(7.78)	34.7	37.3	33.5
Average fertilisation										
With	37.0				33.6					
Without	37.6				33.4					
Average inoculation										
With	35.6				32.3					
Without	38.9				34.7					
LSD cultivars	1.26				2.29					
LSD fertilisation	0.86				0.95					
LSD inoculation	0.75				2.89					

§Percentages of reduction relative to the non inoculated control are given in parenthesis

Table 6.9. Means of test weight (kg/hl) for six wheat cultivars under two N-fertilisation conditions and two inoculation treatments with *Mycosphaerella graminicola*.

	1996				1997					
	With fertilisation		Without fertilisation		With fertilisation		Without fertilisation			
Cultivar	With inoculation	Without inoculation	With inoculation	Without inoculation	With inoculation	Without inoculation	With Inoculation	Without inoculation	Average 1996	Average 1997
	kg hl^{-1}									
Buck Ombú	72.5(2.68)§	74.5	74.4(0.50)	74.0	75.2(1.05)	76.0	74.5(3.50)	77.2	73.8	75.8
Don Ernesto	75.0(0.54)	74.6	73.7(0.50)	73.3	78.7(0.38)	79.0	77.6(2.02)	79.2	74.0	78.6
Klein Centauro	73.4(1.94)	72.0	71.4(0.56)	71.0	78.2(1.76)	79.6	78.4(0.00)	78.4	72.0	78.7
Klein Dragón	72.8(1.22)	73.7	69.7(3.99)	72.6	77.3(1.53)	78.5	76.4(1.31	78.4	72.2	77.6
ProINTA Federal	74.9(2.35)	76.7	73.4(4.80)	77.1	76.0(2.06)	77.6	78.2(1.14)	79.1	75.5	77.7
ProINTA Isla Verde	68.2(4.35)	71.3	68.5(4.06)	71.4	73.6(0.55)	73.2	69.4(5.06)	73.1	69.9	72.3
Average cultivars	72.8(1.36)	73.8	71.8(1.91)	73.2	76.5(1.16)	77.4	75.7(2.32)	77.5	72.9	76.8
Average fertilisation										
With	73.3				76.9					
Without	72.5				76.7					
Average inoculation										
With	72.3				76.1					
Without	73.5				77.4					
LSD cultivars	1.65				1.11					
LSD fertilisation	1.95				0.99					
LSD inoculation	0.60				1.70					

§ Percentages of reduction relative to the non-inoculated control are given in parenthesis.

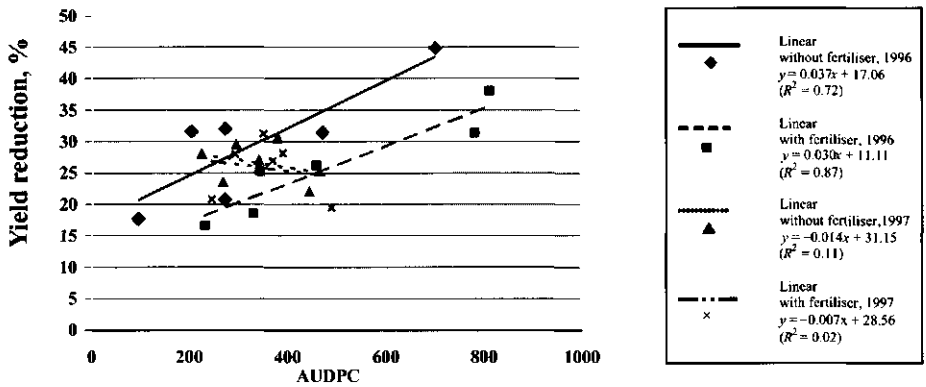

Fig. 6.3. Relationship between AUDPC (area under disease progress curve) of septoria tritici blotch in six wheat cultivars and the reduction in yield per hectare caused by the disease in two fertilisation conditions in 1996 and 1997.

under natural infections caused by septoriosis and rusts, Howard et al. (1994) found important year × fungicide interactions in such a way that yield, EPM^2 and kernel weight were increased by fungicide applications only in some years.

In this study, N-fertilisation had no influence on the relative difference in yield and yield components between inoculated and non-inoculated treatments. This happened in spite of the increase in septoria tritici blotch severity found especially in 1996 in the fertilised plots. Leitch and Jenkins (1995) found that yield increased more when fungicides were applied in fertilised conditions, mainly based on the increase in kernel weight. Johnston et al. (1979) analysed kernel weight and grain yield and for both traits found a higher increase at higher N-conditions when fungicides were applied in one of the years. However, this increase in yield corresponded with a decrease in septoria tritici blotch severity for that year. In both studies, yield and yield components decreased under the effect of *M. graminicola* when the dosage of N was increased. In our study, yield, EPM^2 and KPE were increased by fertilisation also in the inoculated treatment. Thousand-kernel weight was not increased due to the compensation between yield components.

High regression coefficients between AUDPC values and yield reduction due to *M. graminicola* for both N-treatments were found under conducive conditions (1996). However, in 1997, these coefficients were not significant. This was especially due to the behaviour of K. Centauro and K. Dragón. In 1997, these cultivars had increased AUDPC values, but showed similar yields reductions to those recorded in 1996. This indicates the presence of tolerance in these cultivars. Klein Centauro was the latest

maturing cultivar in both years; however as the evaluations were done by the time each cultivar reached the same developmental stage, its reaction could be attributed to a real mechanism of tolerance. Zuckerman and Eyal (1997) indicated the presence of tolerance to *M. graminicola* on some wheat cultivars and showed that photosynthesis in the remaining green tissues of the tolerant cultivars was higher than in non-infected cultivars.

Further study will be necessary to elucidate the behaviour of K. Centauro and to find an explanation for similar reductions in yield between fertilised and non-fertilised treatments. Fertilisation in our research was either 0 or 100 kg ha^{-1} in 1996 under conducive conditions and either 0 or 150 kg ha^{-1} in 1997. Nitrogen doses used in our work fell within the range applied in the growing wheat area in Argentina. Johnston et al. (1979) and Leitch and Jenkins (1995) used doses from 80 to 160 and 100 to 300 kg N ha^{-1}, respectively. With our N-doses and for the environmental conditions of these experiments, percentages of severity for the two upper leaves ranged from 20 to 70% and for the flag leaf between 0.53 and 55% at GS 82-84. A possible explanation would be that the high N-content in the remaining green tissues of the fertilised treatment could compensate for the reduction in photosynthetic area due to necrosis at least when the difference between both treatments was not too high. In addition, higher doses of fertilisers used in other studies could lead to a lower response at extreme N-application rates, particularly when the crop is affected by *M. graminicola.* This may cause the decrease in yield and yield components under inoculation at these N-application rates, determining higher differences between inoculated and non-inoculated treatments under fertilisation.

From our results, it can be concluded that in spite of higher AUDPC values of septoria tritici blotch under conditions conducive to epidemics of *M. graminicola* and N-applications, no significant differences in yield, yield components and TW can be expected at medium fertilisation levels.

CHAPTER 7

General discussion

Introduction

The increased economic importance of septoria tritici blotch in wheat can be mainly attributed to the predominance of susceptible or moderately susceptible cultivars and the increment of cropping systems characterised by poor management of crop residues and increased N fertilisation.

New sources of resistance and adequate management of the disease are required. In this thesis, new sources of resistance were investigated determining the chromosomal location in some of these resources. Genetic variation for resistance and cultivar × isolate interactions in the adult stage were studied in a broad spectrum of Argentinean wheat cultivars. Associations between heading date, plant height and the resistance were also investigated and the effect of N fertilisation on the susceptibility of wheat to septoria tritici blotch and its influence on yield and yield components were analysed.

The general discussion of this thesis gives an overview of the increase in insight obtained in the effects of genetic and morphophysiological factors on the resistance to septoria tritici blotch and in the modifying environmental and cultural factors. Major points emerging from this thesis are reviewed and further discussed. Opportunities for future direction of research are also elaborated.

Genetic resistance

Genetic resistance is the most cost-effective and environmentally appropriate technique for crop disease management. New sources of resistance are required as only a few wheat varieties currently available have adequate levels of resistance.

In the context of looking for new sources of resistance and more precise understanding of location of genes for resistance to septoria tritici blotch, several experiments were carried out. Once chromosomes carrying resistance have been identified, recombinant chromosome lines can be developed to map the resistant genes.

High levels of resistance were identified in the seedling stage in a Synthetic 6x (*Triticum diccocoides* × *Triticum tauschii*) to all isolates tested, including seven Argentinean and three Dutch isolates (Chapter 2). Some other accessions showed acceptable levels of resistance in the seedling stage (< 25% of necrosis with some isolates). Accessions with resistance or moderate resistance to many isolates included *T. spelta*, Cappelle-Desprez, Mara and Hobbit Sib (resistant to 7 out of 10 isolates) and Cheyenne and Bezostaya (resistant to 4 out of 10 isolates). For the Synthetic 6x, resistance to 9 out of 10 isolates was found by Arraiano et al. (2001). In their experiment,

the Synthetic 6x was only susceptible to isolate IPO 92006 from Portugal.

Our results showed that in the adult stage the Synthetic 6x and Cheyenne were resistant or moderately resistant to all of the four isolates tested; Chinese Spring was susceptible or moderately susceptible and Cappelle-Desprez and *T. spelta* showed variable results.

We showed by analysis of the Chinese Spring (Synthetic 6x) substitution series with the Argentinean isolates IPO 92067 and IPO 93014 that a gene or genes on Synthetic 6x chromosome 7D controlled the resistance, found in that material during the seedling stage. In adult plants, 7D also showed to carry genes for resistance to isolate 92067 but not to isolate 93014. Some other chromosomes such as 5A and 5D showed to carry quantitative resistance mainly expressed in the adult stage to both isolates. Other chromosomes were effective against only one isolate. Arraiano et al. (2001), using isolates from The Netherlands and Portugal, found resistance in the 7D chromosome on detached seedling leaves and in the adult stage, and the gene *Stb5* was mapped near the centromere. Resistance on 7D was found to be monogenic and race-specific and *Stb5* was not effective against IPO 92006.

Synthetic wheats are important in breeding programmes because they are relatively easy to cross with common wheats and their resistance can be introgressed into agronomically acceptable genotypes and combined with other resistances. Several accessions of *Triticum tauschii,* the donor of the D genome in Synthetic 6x, carry genes for resistance to leaf rust, powdery mildew, greenbug, Russian wheat aphids, Hessian fly, soil-borne mosaic virus, and stagonospora nodorum blotch (Kerber and Dyck, 1969; Gill et al., 1986; Cox et al., 1992; May and Lagudah, 1992; Murphy et al., 2000; Smith et al., 2000). In the other materials studied (Cheyenne in the seedling and the adult stages and Cappelle-Desprez and *T. spelta* in the seedling stage), levels of resistance found were not as high as in Synthetic 6x and several chromosomes showed quantitative resistance.

Quantitative resistance against both isolates (IPO 92067 and 92064) was found on chromosome 1B of Cheyenne in the seedling stage and on 1B and 5D in the adult stage. Also some other chromosomes showed to carry quantitative resistance in the adult stage for any of these two isolates. The chromosomal location in Cappelle-Desprez and *T. spelta* was studied in the seedling stage only. Chromosomes 2B, 3A and 3B from Cappelle-Desprez showed to carry resistance against both isolates. Some more chromosomes had alleles that were effective against IPO 93014. Chromosome 7D from *T. spelta* proved to carry genes for resistance to IPO 92067 in the seedling stage. Minor gene effects against both isolates were shown on chromosome 6D whereas some other chromosomes showed some effects against one of the two isolates.

Genetic resistance of 50 Argentinean cultivars was also studied in field experiments for two years (Chapter 3). Variation in quantitative resistance was found with a virulent Argentinean isolate (IPO 99013). Two cultivars showed low values of necrosis and pycnidial coverage in the seedling and adult stages (Klein Volcán and Klein Estrella and Klein Dragón) whereas some other cultivars showed good levels of resistance either in the seedling (Buck Chambergo, ProINTA Puntal, Klein Don Enrique, Buck Panadero) or in the adult stage (Cooperación Millán, Granero INTA). Arama (1996) also found cultivars with combined resistance in the seedling and adult stages, whereas some other cultivars were resistant either in the seedling stage or in the adult stage. Kema and Van Silfhout (1997) also showed that not all isolates responded similarly to seedling and adult plant infection. When we tested a set of 16 of the 50 cultivars with 7 isolates in the adult stage, specific cultivar × isolate interactions were present. Although most previous studies have concentrated on the cultivar × isolate interactions in the seedling stage (Eyal et al., 1985; Perelló et al., 1991; Ahmed et al., 1995; Ballantyne and Thomson, 1995; Kema et al., 1996a, b), a few of them also reported interactions in the adult stage (Kema and Van Silfhout, 1997; Brown et al., 2001). In our studies, Klein Dragón and Klein Volcán showed acceptable levels of resistance to all of the isolates tested. Although more isolates should be used to know if overall resistance is present in these cultivars, our results indicate that they carry quantitative resistance factors to several isolates.

Modifying effects of heading date, plant height and weather conditions on the resistance to septoria tritici blotch

Our results showed no genetic associations between the resistance to *Mycosphaerella graminicola* and heading date or plant height within a broad spectrum of Argentinean cultivars tested with one virulent Argentinean isolate (Chapter 3). Associations between pycnidial coverage percentage and days to heading were positive or negative depending on whether weather conditions before the evaluations were more conducive to the development of the disease in late or early cultivars, respectively. Negative associations with plant height were only present in 1998 when weather conditions were less conducive to the development of the disease than in 2000. Unconducive conditions and longer distances between leaves in tall cultivars could have reduced the rain-splash dispersal of pycnidiospores thus causing this negative association. Associations with plant height could also depend on the presence of the teleomorphic stage and the importance of the ascospore release during the growth of the wheat crop. The air-borne dispersal of ascospores could reduce the effect of plant height in the

expression of the disease. The presence of the teleomorphic stage during the whole growing period has been reported in Argentina (Cordo et al., 1990, 1999). No associations with heading date or plant height were found when resistance was assessed in controlled conditions and plants were inoculated in the seedling stage or at the same adult growth stage for all cultivars. These results strongly suggest that within this group of materials environmental and epidemiological factors are much more important than genetic factors in determining associations between resistance to septoria tritici blotch, plant height and heading date.

Epidemiological associations between heading date, plant height and the resistance were also studied in near isogenic lines differing in genes for reduction in plant height (*Rht*) or insensitivity to photoperiod (*Ppd*) (Chapter 4). For the average of two years, isogenic lines of the cultivar Mercia carrying *Rht*3 (Tom Thumb) and *Rht*12 (Karkagi 522) caused the highest reduction in plant height and showed higher necrosis percentages than the Mercia control. Lines of the cultivar Cappelle-Desprez carrying *Rht*3 from Tom Thumb, which showed strongly reduced plant height, showed higher necrosis percentage than the Cappelle-Desprez control. Lines of the Mercia cultivar carrying genes *Ppd*1 (Mara) and *Ppd*1 (Ciano 67) and lines of Cappelle-Desprez carrying *Ppd*1 (Mara) and *Ppd*2 (Chinese Spring), which progressed more rapidly to heading, also showed a lower necrosis percentage than the control. In these experiments, the lines with *Rht* genes, showing the highest necrosis percentage were much shorter than their respective controls with differences in height to flag leaf between 39.7 and 41.3 cm. Negative associations between necrosis percentage caused by septoria tritici blotch and plant height were more consistent over environments than in the experiment with 50 cultivars (Chapter 3) where the largest difference in height to flag leaf was only 25 cm. The positive association between susceptibility and heading date can be explained by weather conditions that predisposed the development of the disease in the late cultivars. In Chapter 5, the relationship between heading date and resistance to septoria tritici blotch was also evaluated in a two years experiment. Those associations were negative in 1996 and positive in 1997 depending also on weather conditions.

We can conclude that no genetic association between resistance, heading date and plant height can be seen within the broad spectrum of Argentinean cultivars studied. Associations between resistance and heading date were because of weather conditions predisposing the development of the disease in late or early cultivars. Associations between susceptibility and shortness were evident when differences in plant height of the lines were high or when the weather conditions were not conducive to the disease delaying the progress to the upper leaves in tall cultivars. This indicates that moderately short cultivars could express high levels of resistance. True resistance

can be assessed evaluating septoria tritici blotch at the same development stage in areas where weather conditions are not very variable at the time of evaluations for early and late cultivars. When weather conditions are variable, data should be corrected based on heading date.

Influence of N fertilisation on the expression of resistance to septoria tritici blotch and on the yield and yield components

N fertilisation caused in general an increase in the development of septoria tritici blotch in conducive weather conditions (Chapter 5). Similar results were found by Gheorghies (1974), Prew et al. (1983), Broscius et al. (1985), Hayden et al. (1994), Howard et al. (1994) and Leitch and Jenkins (1995). Some other experiments showed contradictory results when weather conditions were not conducive to the development of the disease or soil conditions were not suitable for the best utilisation of additional N. We used rates that are fairly high for extensive wheat cultivation (100-150 kg N ha^{-1}). Our aim was to study the effect of these fairly high rates on the progress of the disease and in future studies to use several rates to evaluate the response to N as a quantitative variable. However, the increment of the severity of the disease under these high rates, although significant, was not high. Spore germination is enhanced by longer periods of leaf wetness (Holmes and Cohloun, 1974), so a denser canopy resulting from increased N rate (Seligman et al., 1983) should increase spore germination.

Despite the increase in the area under disease progress curve under N fertilisation when the environment was conducive, the percentage of reduction in yield, yield components and test weight by the disease was similar under both N fertilisation conditions suggesting tolerance mechanisms (Chapter 6). A possible explanation would be that the high N content in the remaining green tissues of the fertilised treatment could compensate for the extra reduction in photosynthetic area due to necrosis. This effect is likely to happen especially when differences in severity of the disease among N treatments are not too large. The mechanisms causing tolerance in general remains vague (Zuckerman et al., 1997).

Utilisation of the results

Chromosomal location of resistance to septoria tritici blotch in substitution lines of wheat allowed us to detect major and minor effects on different chromosomes of several materials. We also identified that in some cases resistance was conditioned by

factors located on the same chromosomes in the seedling stage and the adult stage, whereas in some other cases different chromosomes contributed to the resistance. This knowledge will facilitate the location of genes for resistance through the development of recombinant, double haploid or introgression lines for those specific chromosomes. On the 7D chromosome, a gene for resistance was located (Arraiano et al., 2001). Knowledge about precise location of genes and the finding of markers linked to them are important tools for selection in breeding programmes without the need of inoculation with the pathogen.

Areas for further research

The presence of genetic associations between resistance, heading date and plant height may depend on the genetic materials used. However, results of this work demonstrated that within a wide spectrum of materials it would be possible for breeders to select for short and early heading cultivars with resistance to the septoria tritici blotch. The use of molecular markers will also be useful for identifying associations between heading date, plant height and resistance to *Mycosphaerella graminicola*. Our further research allowed us to determine some QTLs for resistance in the seedling and adult stages (unpublished results of Simón et al., 2003) in recombinant lines of a Synthetic 6x (*T. tauschii* × Altar 84) × Opata 85. These QTLs were found in regions which do not coincide with those where QTLs for flowering time were previously mapped for the same population (Börner et al., 2002). This also suggests that at least in some materials those traits are not genetically linked.

Tolerance of wheat cultivars is another important point for further investigation. Although it is clear that some cultivars have less reduction in yield than expected from the severity of the disease caused by septoria tritici blotch, tolerance mechanisms are not well understood.

Further work will focus on molecular marker analysis to investigate resistance and incorporation of this resistance into materials with good agronomic characteristics.

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Summary

Breeding objectives are focused on developing disease resistant germplasm with high and stable yields across a wide range of environments. Incorporating durable resistance is a priority since breeding well-adapted germplasm with stable yields without adequate resistance against the major diseases would be impossible. Septoria tritici blotch, caused by *Mycosphaerella graminicola* (Fuckel) Schroeter (anamorph *Septoria tritici* Rob. ex Desm.) is a major disease in several wheat growing areas in the world which causes important yield losses. Genetic resistance is the most cost-effective and environmentally appropriate technique for crop disease management. New sources of resistance are required as only a few varieties currently available have adequate levels or resistance. Location of resistance in synthetic wheats is interesting because they are relatively easy to cross with common wheats and their resistance can be introgressed into agronomically acceptable genotypes and combined with other resistances. Chromosomal location of the resistance is the step preceding the development of recombinant chromosome lines and mapping genes.

Higher levels of resistance are supposed to be genetically or epidemiologically linked to late heading and tallness. The presence of genetic linkages can complicate the breeding for early heading, short cultivars resistant to septoria tritici blotch. It is also necessary to know to what extent resistance is present against a wide spectrum of isolates and whether that resistance is expressed at all stages of plant development. Furthermore, some cultural practices, such as N-fertilisation may modify the expression of the disease. Also the relation between disease response and yield loss is not fully clarified.

The aim of this thesis was to look for new sources of resistance, identifying the chromosomal location of that resistance in some materials and to study the effects of morphophysiological and environmental factors that may modify the expression of that resistance.

Several materials with resistance in seedling stage to septoria tritici blotch were identified using a set of 10 isolates of the fungus (Chapter 2). Synthetic 6x (*Triticum dicoccoides* × *Triticum tauschii*) was resistant to all isolates. *T. spelta*, Cappelle-Desprez, Hobbit Sib and Mara were resistant to 7 out of 10 isolates. Bezostaya and Cheyenne were resistant to four isolates. Resistance was also found in the adult stage in some of these materials with some of the isolates. Chromosomal location was investigated in four sets of substitution lines in the seedling stage (Synthetic 6x, Cheyenne, Cappelle-Desprez and *T. spelta* in Chinese Spring) and in two sets in the adult stage (Synthetic 6x and Cheyenne in Chinese Spring). Isolates IPO 92067 and

IPO 93014 were used for all sets except for Cheyenne where IPO 92067 and IPO 92064 were used. The most consistent effects were found in Synthetic 6x which showed to carry genes for resistance on chromosome 7D in the seedling and adult stages to isolate IPO 92067 and in the seedling stage to isolate IPO 93014. Chromosomes 5A and 5D also showed to carry minor gene effects in the adult stage for both isolates. Chromosome 1B from Cheyenne in the seedling stage and in the adult stage and chromosome 5D in the adult stage showed levels of resistance to both isolates similar to the resistance parent or better than the susceptible parent did. For Cappelle-Desprez, chromosomes 2B, 3A and 3B showed to carry minor gene effects for both isolates in the seedling stage. For *T. spelta*, chromosome 6D carried genes for resistance for both isolates and 7D for isolate IPO 92067 in the seedling stage. Some other chromosomes for the different sets showed to carry resistance to one of the isolates.

Variation in quantitative resistance to a virulent Argentinean isolate (IPO 99013) was found in a set of 50 cultivars. Cultivars Klein Volcán and Klein Estrella and Klein Dragón showed good levels of resistance in the seedling stage and the adult stage, whereas some other cultivars showed good levels of resistance either in the seedling stage (Buck Chambergo, ProInta Puntal, Klein Don Enrique, Buck Panadero) or in the adult stage (Cooperación Millán, Granero Inta). When a set of 16 of these cultivars were tested with 7 isolates in the adult stage, specific cultivar × isolate were found, although Klein Dragón and Klein Volcán showed acceptable levels of resistance to all seven isolates (Chapter 3).

Our results showed no genetic associations between the resistance to *Mycosphaerella graminicola* and heading date or plant height within a broad spectrum of Argentinean cultivars tested with one virulent Argentinean isolate (Chapter 3). When the disease severity was evaluated at the same developing stage in all cultivars, associations depended on how weather conditions predisposed the disease development in early or late cultivars. Negative linear or non-linear correlations with height in this set of cultivars were only significant when weather conditions were less conducive to the disease development. No associations with heading date or plant height were found when resistance was assessed in controlled conditions and plants inoculated in the flag leaf stage. Isogenic lines of the cultivar Mercia carrying *Rht*3 and *Rht*12 and isogenic lines of the cultivar Cappelle-Desprez carrying *Rht*3 showed the highest reductions in plant height (between 39.7 and 41.3 cm) and the highest values of susceptibility to septoria tritici blotch. Isogenic lines of both cultivars carrying *Ppd* genes for insensitivity to photoperiod showed the shortest heading time and the lowest values of severity. Plant height was negatively associated with necrosis percentage and heading date showed some positive associations which can be attributed to weather

conditions being more conducive to the development of the disease in the late lines (Chapter 4). Negative or positive associations between resistance and heading date were found in a set of six Argentinean cultivars grown in 1996 and 1997. These associations also depended on weather conditions (Chapter 5).

Generally, nitrogen fertilisation caused an increase in the development of the disease under conducive weather conditions, but cultivar × N-fertilisation interactions proved to be significant (Chapter 5). Despite the increase of the area under disease progress curve under N fertilisation when environment was conducive, the percentage of reduction in yield, yield components and test weight was similar in N fertilised and non-fertilised conditions suggesting the presence of tolerance mechanisms (Chapter 6).

The contribution of the thesis to the existing knowledge and areas for further research are discussed in Chapter 7. Identification of chromosomes carrying resistance genes to septoria tritici blotch facilitates the location of genes for resistance through the development of recombinant or introgression lines for those particular chromosomes. We are actually testing introgression lines with the 7D and 5D chromosomes of Synthetic 6x in Chinese Spring to map the resistance genes. Knowledge about precise location of resistance genes and the finding of markers linked to them will allow breeders to select for resistance without inoculating crops with the pathogen and avoiding environmental effects on the expression of septoria tritici blotch.

Although the presence of genetic associations between resistance, heading date and plant height may depend on the genetic materials used, results of this work demonstrated that within a wide spectrum of materials it would be possible for breeders to select for short and early heading cultivars with resistance to the disease. The use of molecular markers will also be useful to identify associations between heading date, plant height and resistance to *Mycosphaerella graminicola*. Our further research allowed us to identify in recombinant lines of a Synthetic 6x (*T. tauschii* × Altar 84) × Opata 85 some QTLs accounting for variation in resistance to septoria tritici blotch in the seedling and adult stages. These QTLs did not coincide with the regions where QTLs for flowering time were previously mapped for the same population, indicating that at least within some germplasm these traits are not linked.

Tolerance of wheat cultivars to septoria tritici blotch is another topic for further investigation. Although it is clear that some cultivars suffer less from reduction in yield than expected based on the severity of septoria leaf blotch, the mechanisms of this tolerance is not well understood. Further work will focus on molecular marker analysis to investigate resistance and to incorporate this resistance into materials with good agronomic characters.

Resumen

Los objetivos del mejoramiento del trigo están concentrados en el desarrollo de germoplasma resistente a enfermedades, con rendimientos estables a través de un amplio rango de ambientes y buena calidad industrial. La incorporación de resistencia durable es prioritaria, ya que no es posible obtener germoplasma con rendimientos estables sin resistencia adecuada a las principales enfermedades. La mancha de la hoja del trigo, causada por *Mycosphaerella graminicola* (Fuckel) Schroeter (anamorfo *Septoria tritici* Rob. ex Desm.) es una enfermedad importante en diversas áreas de producción de trigo en el mundo que causa importantes pérdidas de rendimiento.

La resistencia genética es la herramienta con menor relación costo-beneficio y más adecuada para la protección ambiental para el manejo de esta enfermedad. Se requieren nuevas fuentes de resistencia, ya que son pocos los cultivares actuales que tienen adecuados niveles de resistencia. La localización de la resistencia en trigos sintéticos obtenidos del cruzamiento de especies tetraploides y diploides del género *Triticum* es interesante, ya que estos trigos son relativamente fáciles de cruzar con trigos comunes y su resistencia a diversas enfermedades y plagas puede ser incorporada en genotipos agronómicamente aceptables. La localización cromosómica de la resistencia es el paso previo al desarrollo de líneas recombinantes cromosómicas que faciliten el mapeado de genes.

El estudio de la variabilidad genética de cultivares actuales para resistencia y su respuesta a diferentes aislamientos del patógeno y el conocimiento de la expresión de la resistencia en distintos estadios del cultivo es también de utilidad para la identificación de fuentes de germoplasma. Se ha mencionado que la resistencia a la mancha de la hoja del trigo está genética o epidemiológicamente asociada a mayor altura y longitud de ciclo de los cultivares. La presencia de asociaciones genéticas puede dificultar la obtención de cultivares de baja altura y de ciclo corto con adecuada resistencia al patógeno. Además algunas prácticas culturales como la fertilización nitrogenada pueden modificar la expresión de la resistencia, sin resultar claro si existe correlación entre la severidad y las pérdidas de rendimiento producidas por la enfermedad.

Se identificaron diversos materiales con resistencia en plántula a la mancha de la hoja del trigo utilizando un set de 10 aislamientos (Capítulo 2). El trigo sintético hexaploide obtenido del cruzamiento de *Triticum dicoccoides* × *Triticum tauschii* fue resistente a todos los aislamientos. *Triticum spelta*, Cappelle-Desprez, Hobbit Sib y Mara fueron resistentes a 7 aislamientos y Bezostaya y Cheyenne fueron resistentes a 4 aislamientos. También se detectó resistencia en estado adulto en algunos materiales.

Se investigó la localización cromosómica de la resistencia en 4 sets de líneas de sustitución en estado de plántula (Synthetic 6x, Cheyenne, Cappelle-Desprez y *Triticum spelta* en el cultivar susceptible Chinese Spring) y en dos sets en estado adulto (Synthetic 6x y Cheyenne en Chinese Spring). Se utilizaron los aislamientos IPO 92067 and IPO 93014 para los set de Synthetic 6x, Cappelle-Desprez y *T. spelta* en Chinese Spring y los aislamientos IPO 92067 e IPO 92064 para el set de Cheyenne en Chinese Spring Los efectos más consistentes de localización cromosómica se encontraron en Synthetic 6x cuyo cromosoma 7D demostró poseer genes de resistencia al patógeno con ambos aislamientos en estadio de plántula y en estado adulto frente al aislamiento IPO 92067. Los cromosomas 5A y 5D también evidenciaron poseer resistencia cuantitativa en estado adulto con ambos aislamientos. Cheyenne demostró poseer resistencia en el cromosoma 1B de Cheyenne en plántula y estado adulto y el cromosoma 5D en estado adulto. Cappelle-Desprez evidenció poseer factores de resistencia en los cromosomas 2B, 3A and 3B en estado de plántula con ambos aislamientos. Para *Triticum spelta* el cromosoma 6D demostró poseer genes de resistencia para ambos aislamientos en estado de plántula y el 7D para el aislamiento IPO 92067. Otros cromosomas evidenciaron poseer resistencia cuantitativa para alguno de los aislamientos.

Se encontró variabilidad para resistencia cuantitativa entre 50 cultivares argentinos de trigo que representan un amplio espectro de los cultivares liberados en Argentina hasta 1998 frente al aislamiento argentino IPO 99013. Los cultivares Klein Volcán y Klein Estrella y Klein Dragón presentaron buenos niveles de resistencia en plántula y estado adulto en tanto que otros cultivares demostraron poseer resistencia en estado de plántula (Buck Chambergo, ProINTA Puntal, Klein Don Enrique, Buck Panadero) o en estado adulto (Cooperación Millán, Granero INTA). Cuando 16 de estos cultivares fueron inoculados con 7 aislamientos en estado adulto, se encontraron interacciones cultivares × aislamientos. Sin embargo Klein Volcán y Klein Dragón manifestaron aceptables niveles de resistencia con todos ellos (Capítulo 3).

No se encontraron asociaciones genéticas entre la resistencia a la mancha de la hoja del trigo con el ciclo a espigazón o la altura de planta dentro del set de 50 cultivares inoculados con el aislamiento IPO 99013. Evaluando la enfermedad en el mismo estado fenológico en todos los cultivares, las asociaciones dependieron de la forma en que las condiciones ambientales eue favorecieron el desarrollo de la enfermedad en cultivares de ciclo largo o corto. Sólo se evidenciaron asociaciones negativas entre la resistencia y la altura de planta cuando las condiciones ambientales fueron inapropiadas para el desarrollo de la enfermedad. Las líneas isogénicas de los cultivares Mercia con los genes de reducción de altura de planta *Rht*3 y *Rht*12 y las líneas isogénicas del cultivar Cappelle-Desprez con el gen *Rht*3 presentaron las

mayores reducciones en altura de planta (entre 39.7 y 41.3 cm) y los valores de severidad más altos de mancha de la hoja. Las líneas isogénicas de ambos cultivares con genes de insensibilidad al fotoperíodo presentaron un acortamiento del ciclo a espigazón y los valores más bajos de severidad. La altura de planta estuvo generalmente asociada negativamente con el porcentaje de necrosis y el ciclo a espigazón presentó asociaciones positivas que pueden atribuirse a condiciones ambientales más predisponentes para el desarrollo de la enfermedad en las líneas de ciclo más largo (Capítulo 4). También se encontraron asociaciones negativas o positivas entre la resistencia y el ciclo a espigazón en un set de seis cultivares argentinos en dos experimentos a campo en 1996 y 1997. Estas asociaciones también dependieron de condiciones ambientales (Capítulo 5).

La fertilización nitrogenada provocó un incremento en la severidad de la enfermedad en condiciones ambientales predisponentes para su desarrollo, aunque existieron interacciones cultivar fertilización (Capítulo 5). A pesar del incremento en el área bajo la curva de progreso de la enfermedad cuando el ambiente fue predisponente, el porcentaje de reducción en rendimiento, componentes del rendimiento y peso hectolítrico fue similar en los tratamientos con fertilización y sin fertilización nitrogenada sugiriendo la presencia de mecanismos de tolerancia (Capítulo 6).

En el capítulo 7 se discute la contribución de los resultados al conocimiento existente y áreas para futuras investigaciones. La identificación de cromosomas con resistencia a la mancha de la hoja del trigo facilita la localización de genes de resistencia a través del desarrollo de líneas recombinantes o líneas de introgresión para dichos cromosomas. El conocimiento de la localización precisa de genes y marcadores ligados a los mismos es una herramienta importante para la selección en programas de mejoramiento genético sin la necesidad de realizar inoculaciones con el patógeno y evitando la influencia ambiental en la expresión de la resisencia. Aunque la presencia de asociaciones genéticas entre la resistencia, ciclo a espigazón y altura de planta puede depender del germoplasma utilizados, los resultados de este trabajo demostraron que dentro de un amplio espectro de materiales es posible para los mejoradores seleccionar cultivares de baja altura y ciclo corto con resistencia a la enfermedad. El uso de marcadores moleculares también es de utilidad para identificar asociaciones entre la resistencia, ciclo a espigazón y altura de planta. Nuestros trabajos posteriores permitieron determinar en líneas recombinantes de Synthetic 6x (*T. tauschii* × Altar 84) × Opata 85 algunos QTLs que no coincidieron con las regiones donde se maperaron previamente QTL para ciclo a espigazón y altura en la misma población. Esto también indica que no existen ligamientos genéticos entre estos caracteres, al menos en algunos genotipos.

La tolerancia de los cultivares de trigo a la enfermedad es otro aspecto importante para ulteriores investigaciones. Aunque es claro que algunos cultivares tienen una menor reducción en rendimiento que lo esperado de acuerdo a la severidad que presentan, las razones de este mecanismo no han sido explicadas. Nuestro posterior trabajo se concentrará en la localización de marcadores para resistencia a la mancha de la hoja y su posterior incorporación en materiales con adecuadas características agronómicas.

Samenvatting

Veredeling is gericht op het ontwikkelen van genetisch materiaal dat ziekteresistent is en dat hoge en stabiele opbrengsten geeft in ecologisch diverse milieus. De eerste prioriteit is het inbouwen van duurzame resistentie. Het is immers onmogelijk om breed aangepast genetisch materiaal met stabiele opbrengsten te produceren zonder afdoende resistentie tegen de belangrijkste ziekten en plagen.

Mycosphaerella graminicola (Fuckel) Schroeter (anamorf *Septoria tritici* Rob. ex Desm.) is de veroorzaker van de bladvlekkenziekte in tarwe. De bladvlekkenziekte is één van de belangrijkste ziekten in verschillende gebieden van de wereld waar tarwe op grote schaal geteeld wordt. De ziekte veroorzaakt aanzienlijke opbrengstverliezen. Genetische resistentie is economisch het meest effectief en milieutechnisch de meest geschikte manier om de ziekte te beheersen. Aangezien er nu nog slechts weinig rassen beschikbaar zijn die over voldoende resistentie tegen de bladvlekkenziekte beschikken, zijn nieuwe bronnen van resistentie nodig. Het is interessant om de locatie van de resistentie in 'synthetics' van tarwe vast te stellen, aangezien dergelijke synthetics eenvoudig te kruisen zijn met de gangbare tarwes en omdat de resistentie van dergelijke synthetics eenvoudig kan worden ingebracht in genotypen die reeds over de gewenste agronomische eigenschappen beschikken. Bovendien kan zo de resistentie tegen de bladvlekkenziekte eenvoudig worden gecombineerd met resistenties tegen andere ziekten en plagen. Het vaststellen op welke chromosomen de resistentiegenen gelegen zijn, is de eerste stap op weg naar het ontwikkelen van chromosoom-recombinatielijnen en naar het in kaart brengen van de betrokken genen.

Het is bekend dat grotere resistentie genetisch of epidemiologisch gekoppeld is aan het laat in de aar schieten en aan langere planten. Dergelijke genetische koppelingen kan het veredelen bemoeilijken op rassen die vroeg in de aar schieten, kort zijn en toch resistent zijn tegen de bladvlekkenziekte. Het is ook noodzakelijk meer te weten over de mate van resistentie tegen een breed spectrum aan isolaten van de schimmel en over de vraag of dergelijke resistenties aanwezig zijn in alle stadia van de gewasontwikkeling. Bovendien is bekend dat sommige teeltmaatregelen, zoals stikstofbemesting, het tot expressie komen van de resistentie kunnen modificeren. Tenslotte is ook de relatie tussen de mate van aantasting door de ziekte en het door de ziekte veroorzaakte opbrengstverlies niet voldoende duidelijk.

Dit proefschrift beoogt nieuwe bronnen van resistentie op te sporen, de positie van de resistentie op het genoom vast te stellen en de effecten van morfofysiologische en omgevingsfactoren te bestuderen die de expressie van resistentie tegen de bladvlekkensziekte kunnen modificeren.

Gebruikmakend van 10 isolaten van het pathogeen werd verschillend materiaal geïdentificeerd dat in het zaailingstadium resistent bleek te zijn tegen de bladvlekkenziekte (Hoofdstuk 2). 'Synthetic 6x' (*Triticum dicoccoides* × *Triticum tauschii*) bleek tegen alle isolaten resistent te zijn. *T. spelta* en de tarwerassen Cappelle-Desprez, Hobbit Sib and Mara waren resistent tegen 7 van de 10 isolaten. De rassen Bezostaya en Cheyenne waren resistent tegen vier isolaten. Voor sommige rassen en bij bepaalde isolaten werd ook resistentie in het volwassen stadium gevonden. Gebruikmakend van de isolaten IPO 92067, IPO 93014 en IPO 92064 werd in vier sets van substitutielijnen (Synthetic 6x, Cheyenne, Cappelle-Desprez en *T. spelta* in Chinese Spring) in het zaailingstadium en in twee sets (Synthetic 6x en Cheyenne in Chinese Spring) in het volwassen stadium de positie van de resistentie-eigenschappen op het genoom onderzocht. De meest consistente resultaten werden voor de Synthetic 6x gevonden: Synthetic 6x bleek op chromosoom 7D genen voor resistentie tegen het isolaat IPO 92067 in het zaailing- en in het volwassen stadium en tegen isolaat IPO 90314 in het zaailingstadium te bezitten. Voor beide isolaten bevonden zich op de chromosomen 5A en 5D 'minor genes' voor resistentie in het volwassen stadium. Chromosoom 1B van Cheyenne (voor zowel het zaailing- als het volwassen stadium) en chromosoom 5D (voor het volwassen stadium) resulteerden in resistentieniveaus tegen beide isolaten die vergelijkbaar waren met het resistentieniveau van de resistente ouder, of in elk geval hoger waren dan het resistentieniveau van de vatbare ouder. Voor het ras Cappelle-Deprez bleken de chromosomen 2B, 3A en 3B drager te zijn van 'minor genes' met effecten op beide isolaten in het zaailingstadium. Voor *T. spelta* bleken chromosoom 6D genen te bevatten die zorgen voor resistentie tegen beide isolaten in het zaailingstadium en chromosoom 7D voor resistentie tegen het isolaat IPO 92067 in het zaailingstadium. In beide sets bleken sommige andere chromosomen eveneens genen voor resistentie tegen één van de beide isolaten te bevatten.

In een set van 50 rassen werd variatie in kwantitatieve resistentie aangetroffen tegen een virulent isolaat uit Argentinië (IPO 99013). De rassen Klein Volcán, Klein Estrella en Klein Dragón bleken over hoge niveaus van resistentie te beschikken in zowel het zaailing- als het volwassen stadium. Sommige andere rasssen vertoonden alleen goede resistentie in het zaailingstadium (Buck Chambergo, ProINTA Puntal, Klein Don Enrique, Buck Panadero) of in het volwassen stadium (Cooperación Millán, Granero INTA). Bij het testen van 16 van deze 50 rassen op resistentie tegen zeven isolaten in het volwassen stadium bleek de interactie tussen ras en isolaat significant te zijn. In onze proeven bleken de rassen Klein Dragón en Klein Volcán redelijk resistent tegen alle zeven isolaten te zijn.

De resultaten toonden verder aan dat er geen sprake was van genetische

koppeling tussen de resistentie tegen *Mycosphaerella graminicola* en de datum van het in de aar komen of de planthoogte. Dit gold voor een breed spectrum van Argentijnse rasssen getoetst met één virulent Argentijns isolaat (Hoofdstuk 3). Wanneer de mate van aantasting door de ziekte in voor alle rassen hetzelfde stadium werd bepaald, dan bleek de koppeling af te hangen van de wijze waarop de weersomstandigheden de ontwikkeling van de ziekte in vroege of late rassen voorbeschikten. Negatieve lineaire of non-lineaire correlaties tussen ziekte-aantasting en planthoogte bleken in deze set rassen alleen significant wanneer de weersomstandigheden minder gunstig waren voor de ontwikkeling van de ziekte. Er werden geen koppelingen met het moment van in de aar schieten of planthoogte gevonden wanneer de resistentie werd bepaald onder gecontroleerde omstandigheden en bij planten die in het vlagbladstadium waren geïnoculeerd. Isogene lijnen van het ras Mercia die de genen *Rht*3 en *Rht*12 bevatten en de isogene lijnen van het ras Cappelle-Deprez die het gen *Rht*3 bevatten vertoonden de grootste reducties in planthoogte (tussen 39.7 en 41.3 cm) en de hoogste waarden voor vatbaarheid voor de bladvlekkenziekte. Isogene lijnen van beide rassen met *Ppd* genen voor ongevoeligheid voor daglengte vertoonden een korte tijd tot het in de aar schieten en de laagste waarden voor vatbaarheid. De planthoogte was negatief gecorreleerd met het percentage necrose. Het moment van het in de aar schieten gaf enige positieve correlaties met het percentage necrose. Dit laatste effect kan worden toegeschreven aan de weersomstandigheden die de ontwikkeling van de ziekte in de late lijnen meer bleken te bevorderen (Hoofdstuk 4). Voor een zestal Argentijnse rassen, geteeld in 1996 en 1997, werden negatieve of positieve verbanden gevonden tussen resistentie en datum van in de aar schieten.

In het algemeen gaf een stikstofbemesting onder voor de ziekte gunstige omstandigheden een toename in de ontwikkeling van de ziekte. De interacties tussen ras en stikstofbemesting bleken echter significant (Hoofdstuk 5). Ondanks de toename van de AUDPC (de oppervlakte onder de curve die de toename van de ziekte weergeeft) onder voor de ziekte gunstige weersomstandigheden bleken opbrengst, opbrengstcomponenten en het volumegewicht percentagegewijs in gelijke mate door de ziekte af te nemen in de bemeste en onbemeste behandelingen. Deze waarneming suggereert dat er sprake is van tolerantiemechanismen (Hoofdstuk 6).

In de algemene discussie (Hoofdstuk 7) wordt de bijdrage van het in dit proefschrift beschreven onderzoek aan de bestaande kennis alsmede terreinen voor nader onderzoek beschreven. Na het identificeren van chromosomen die genen dragen die resistentie tegen de bladvlekkenziekte bewerkstelligen, is het eenvoudiger resistentiegenen te localiseren door het ontwikkelen van recombinante of introgressielijnen voor deze specifieke chromosomen. In feite zijn we op dit moment al bezig introgressielijnen te testen waarbij de chromosomen 7D en 5D van Synthetic

6x ingebracht zijn in Chinese Spring om de resistentiegenen in kaart te brengen. Kennis omtrent de preciese locatie van de resistentiegenen en de identificatie van daaraan gekoppelde merkers zal het voor veredelaars mogelijk maken om op resistentie te selecteren zonder de gewasssen met het pathogeen te inoculeren. Zo kunnen bovendien milieu-effecten op de expressie van de bladvlekkenziekte worden omzeild.

Hoewel de aanwezigheid van genetische koppeling tussen resistentie, datum van het in de aar schieten en planthoogte kan afhangen van het gebruikte genetische materiaal, tonen onze resultaten aan dat het mogelijk is binnen een breed spectrum van genetisch materiaal te selecteren op korte, vroeg in de aar schietende met resistentie tegen de bladvlekkenziekte. Het gebruik van moleculaire merkers zal ook nuttig zijn bij het identificeren van koppelingen tussen de datum van in de aar schieten, planthoogte en resistentie tegen *Mycosphaerella graminicola*. Vervolgonderzoek stelde ons in staat om in recombinantlijnen van een Synthetic 6x (*T. tauschii* × Altar 84) × Opata 85 enkele QTLs te identificeren die een deel van de variatie in resistentie tegen de bladvlekkenziekte zowel in het zaailing- als in het volwassen stadium verklaren. Deze QTLs vielen niet samen met de gebieden op het genoom waarop eerder – voor dezelfde populatie – QTLs voor bloeidatum in kaart waren gebracht. Dit geeft aan dat deze eigenschappen in elk geval voor sommig kiemplasma niet is gekoppeld.

Ook tolerantie voor de bladvlekkenziekte bij tarwerassen verdient nader onderzoek. Hoewel bekend is dat sommige rassen minder opbrengstverlies lijden dan verwacht kan worden op basis van de mate van aantasting met de bladvlekkenziekte is nog weinig bekend over de mechanismen van tolerantie. Nader onderzoek zal zich concentreren op de analyse van moleculaire merkers om resistentie nader te onderzoeken en om te pogen resistentiegenen in te brengen in materiaal met een goede cultuurwaarde.

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Supervisor. Dr. Rients Niks.

Curriculum vitae

María Rosa Simón was born on 10 March 1955 in La Plata, Argentina. From 1972 until 1977, she studied for her BSc degree at the former Facultad de Agronomía, Universidad Nacional de La Plata (currently Facultad de Ciencias Agrarias y Forestales, FCAyF, UNLP). In 1977, she joined the Cátedra de Cerealicultura (now Curso Cerealicultura, Departamento de Tecnología Agropecuaria y Forestal, FCAyF, UNLP). Between 1977 and 1988, she worked as teaching and research assistant. In 1988, she was appointed Chief of Practicals. In 1990, she started studying in Wageningen, participating in the XXth International Plant Breeding Course of the International Agricultural Centre. In 1993, she started her MSc in Crop Science, specialisation Plant Breeding, at the former Wageningen Agricultural University (now Wageningen University) and graduated in 1995. From 1996 to 1998, she continued her work at the FCAyF as Adjunct Professor, started writing the project proposal and initiated the research for her PhD. In 1999, she got a research grant from the FOMEC (Fondo Mejoramiento Calidad de la Enseñanza Universitaria) to pursue her PhD at the Department of Plant Sciences, Wageningen University. She carried out experiments in Wageningen, The Netherlands and in Argentina. After graduation she will continue to work for the FCAyF.