Mechanisms of resistance to Fusarium ear blight in winter wheat (*Triticum aestivum* L.).

Thesis

UNRESTRICTED

MECHANISMS OF RESISTANCE TO FUSARIUM EAR BLIGHT IN WINTER WHEAT (*Triticum aestivum* L.)

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To Dad,

Dr P.A Hilton (senior)CGM. Ceng. MRAe.S. M.A.I.A.A

who was recently awarded (1994) an honoury doctorate from Brighton University for services to mechanical engineering.

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ABSTRACT

The addition of anthers from five cultivars of winter wheat to 10μl of a conidial suspension of *F. culmorum* significantly increased conidial germination, germ-tube branching and growth compared with a water control. Although anthers from the cultivars Beaver and Mercia caused significantly more branching of germ tubes of *F. culmorum* than Hussar and Riband, there were no differences between cultivars in % germination and total germ-tube length. The role of anthers in the resistance of wheat cultivars to initial infection by *F. culmorum* is discussed.

Resistance to spread of infection was examined by measuring the % of necrotic spikelets after a single spikelet in the middle of the ear was inoculated with either *F. culmorum* or *F. graminearum*. Significant differences between cultivars were observed in % necrotic spikelets below the point of inoculation 4 weeks after inoculation with *F. culmorum*. Premature bleaching (scalding) occurred in the top half of ears above the point of inoculation and was associated with cultivars showing high levels of necrosis. The levels of necrosis and scalding in these cultivars could not be related to severity of Fusarium Ear Blight (FEB) observed in the field and reasons for this have been discussed.

Field studies revealed that cultivar morphology including total straw height and compactness of ear could also significantly affect the severity of FEB. In 1995/96 field trials artificially inoculated with a mixture of *Fusarium* spp and *Microdochium nivale*, (concentration 2.5 X 10^5 ml^{-1} of water) showed that short strawed cultivars with lax ears had more symptoms of FEB than taller strawed cultivars with dense ears. Among

random F_3 populations derived from cultivars of varying height there was a clear tendancy for tall strawed lines to show less disease symptoms than shorter lines following inoculation suggesting that the relationship between straw height and disease severity had a genetic basis. Monitoring relative humidity at ear height in a short and tall isogenic line of Maris Huntsman revealed no significant differences between these genotypes from GS 65 to GS 85, suggesting that microclimate cannot explain differences in severity between tall and short lines. The implications of these results are discussed in terms of breeding cultivars resistant to FEB of any height and with suitable ear characteristics.

Symptoms of FEB seen on different cultivars of wheat in the field are, therefore, not only due to differences to initial infection in the anthers and spread of necrosis within the ear but also differences in scalding. It has also been shown that a number of morphological characters, including cultivar height, significantly affect severity of FEB.

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Hilton AJ, Jenkinson P, Hollins TW and Parry DW, 1999. Relationship between cultivar height and severity of Fusarium ear blight in wheat. *Plant Pathology* **48,** 202-208.

Hilton AJ, Jenkinson P, Hollins TW and Parry DW. 1998. Resistance to initial infection and colonization by Fusarium ear blight in winter wheat. In: *Proceedings of the 7th International Congress of Plant Pathology, Edinburgh 1998, Vol 3, 3.4.12.*

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Hilton AJ, Jenkinson P, Parry DW and Hollins TW. 1996. Relationship between plant morphology and severity of Fusarium ear blight in eight cultivars of winter wheat. In: *Proceedings of the Brighton Crop Protection Conference - Pests and Diseases, 1996 Vol 2, 419-420.*

PRESENTATIONS

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CHAPTER 1

Literature Review

1.1 General Introduction

Fusarium ear blight (FEB), also known as head blight, is a significant disease of wheat throughout the world. Seventeen pathogenic species have been associated with the disease including: *Fusarium acuminatum, F. anthophilum, F. avenaceum, F. culmorum, F. dimerum, F. equiseti, F. graminearum, F. merismoides, F. oxysporum, F. poae, F. sacchari, F .sambucinum, F. solani, F. sporotrichioides, F .tricinctum, F. verticillioides* and *Microdochium nivale* (formerly *F. nivale*) (Nirenberg, 1981). However, only five main species are considered as significant causal organisms: *F. culmorum, F. graminearum (Gibberella zeae), F. avenaceum (G. avenacea), F. poae* and *M. nivale (Monographella nivalis)*. These pathogens not only affect wheat but also other small grain cereals including barley, oats, rye and triticale (Parry *et al.*, 1995).

The first record of FEB was made in the UK by Smith (1884) who identified the causal organism as *Fusisporium culmorum*. Atanasoff (1924) regarded FEB as a common disease throughout cereal growing areas of the USA. The disease has now been recorded in many countries including Canada (Gordon, 1959), Mexico (Ireta and Gilchrist, 1994), Australia (Francis and Burgess, 1977), Netherlands (Snijders, 1990a), France (Saur and Benacef, 1993), Argentina (Moschini and Fortungno, 1996) and China (Cook, 1981).The predominant species responsible for disease may vary with geographical location. For example, in the continental climate of Manitoba, Canada, a survey of farmers fields revealed that *F. graminearum* was the most prevalent species accounting for 52 % of isolates found on wheat (Wong *et al.*, 1992). In the cooler climate of the Netherlands, *F. culmorum* was the predominant species found (Daamen *et al.*, 1991). In England and Wales, a survey undertaken between 1989-1990 showed that *F. poae*

was the predominant species accounting for 54 % of isolates found on the ear, whilst *M. nivale* showed a greater importance in Scotland, accounting for 53 % of isolates (Polley *et al.*, 1991). In colder regions of Europe *M. nivale* was identified as the principal causal organism (Cassini, 1981).

1.2 Symptoms of FEB

Initial symptoms of FEB on wheat consist of small brown water soaked spots at the base of the outer glumes. Complete necrosis of the outer glumes then occurs before symptoms develop on the rachis and adjacent spikelets. In some cases the healthy part of the ear above the point of infection may bleach readily without passing through the necrotic phase (Atanasoff, 1920). In prolonged warm, humid conditions, white mycelium and pink sporodochia form salmon-coloured encrustations on spikelets, especially at the base of the glumes (Nelson, 1929). Such symptoms are common on susceptible cultivars, such as Virtue (Plate 1.1). Grain from infected ears is characteristically shrivelled in appearance and white or pale pink in colour and have been termed 'tombstone' kernels by Canadian workers (Abramason *et al.*, 1987).

Plate 1.1 Severe necrosis on ears of the susceptible winter wheat cultivar Virtue caused by infection with *Fusarium* species.

1.3 Significance

1.3.1 Effect on yield

In the Yangtse valley in China, FEB caused an estimated 5 -50 % loss in yield, with losses reaching 50 % approximately one year in five (Ireta and Gilchrist, 1994). In a naturally infected trial in India, *F. avenaceum* caused yield losses of between 15 and 29 % (Chaudhary *et al.*, 1990).

Other data has been obtained where plants have been artificially inoculated in the field, at mid anthesis, as part of screening programmes for resistance to FEB. For example, Saur (1991) reported yield reductions of between 6 and 39 % when over 500 wheat genotypes and lines were artificially inoculated with *F. culmorum* and *F. graminearum*. When winter rye breeding lines were artificially inoculated, yield losses of between 27 and 49 % were reported due to *F. culmorum*, and losses of between 38 and 52 % for infection by *F. graminearum* (Miedaner *et al.*, 1993). Snijders and Perkowski (1990) reported reductions in thousand grain weight in winter wheat of between 4 and 22 % following artificial inoculation with *F. culmorum*.

1.3.2 Effect on grain quality

The genus *Fusarium* is one of the most prolific mycotoxin-producing genera (Rotter *et al.*, 1996), producing a large number of compounds belonging to the tricothecene group. In a survey carried out in Manitoba, Canada, on wheat samples suspected of being infected with *Fusarium* species, deoxynivalenol (DON or vomitoxin) was found in 39 out of 48 samples at ≤1.40 mg/kg, and in one sample at 3.65 mg/kg (Abramason *et al.*, 1987). Diacetoxyscirpenol was found in 20 samples at ≤0.08mg/kg, HT-2 toxin

in 10 samples at <0.05 mg/kg, and T-2 toxin in 11 samples at ≤0.20mg/kg. In the UK, *Fusarium* mycotoxins including nivalenol, DON, HT-2 toxin, monoacetotoxyscirenol, diacetoxyscirpanol, 3-acetyldeoxynivalenol and in one incidence zearalenone were detected in the range of 5-100 μg/kg and confirmed in approximately 25 % of wheat samples randomly selected from a large number of survey fields (Polley *et al.*, 1991).

When infected grain is fed to livestock various toxicological and immunotoxic effects may result. Pigs fed grain infected with *Fusarium* have shown reduced food intake (Rotter *et al.*, 1994), damage to the oesophageal part of the stomach (Trenholm *et al.*, 1994) and vaginal prolapses (Long *et al.*, 1982). When poultry were fed grain infected by *F. poae, F. culmorum* or *F. graminearum,* stunted growth and poor feathering resulted (Hoerr *et al.*, 1982). In humans, incidental evidence has indicated a relationship between *F. poae* and *F. sporotrichoides* found in overwintered cereals and the development of Alimentary Toxic Aleukia (Joffe, 1978). The effect of *Fusarium*-contaminated wheat was compared with grain that had been treated with comparable concentrations of laboratory synthesized pure DON on feed intake, body weight gain and appearance and histological characters of a number of organs in pigs (Trenholm *et al.*, 1994). This study showed that although similar effects were found, *Fusarium* contaminated grain caused a greater reduction on feed intake and body weight gain, despite DON being the only mycotoxin found on infected grain. Other factors or mycotoxins that were not detected may, therefore, be important in causing deleterious effect in pigs. *Fusarium* mycotoxins have been widely reviewed by Chelkowski (1989) and the toxicology of DON has been extensively discussed by Rotter *et al.* (1996).

Fusarium infection of wheat can cause deleterious effects on bread quality. For example, Dexter *et al.* (1996) showed that as the proportion of *Fusarium* damaged grains increased in samples of wheat from 1.5 % in cleaned samples to 8 % in damaged samples, so dough became more sticky and the proportion of glutenins in the gluten declined. In this case, *Fusarium* damage was described as those grains looking "lifeless, thin, shrunken and affected by a whitish or pinkish fibrous mould" which, unfortunately, did not take account of those infected grains not showing these symptoms. Contamination of wheat and barley with *Fusarium* spp. is also believed to cause the phenomenon of 'gushing' in the beer making process (Narziβ *et al.*, 1990). Schwarz *et al.* (1996) micromalted fifty barley samples, displaying a range of 0 to 100% seeds infected with *Fusarium,* and found that those samples which were infested with *Fusarium* tended to 'gush'. Levels of deoxynivalenol and ergosterol were found to be strongly correlated with amount of gushing observed (Schwarz *et al.*, 1996).

1.3.3 Effect on seed quality

Infected seed resulting from FEB can provide a primary source of inoculum for the development of seedling blight and foot rot (Duthie and Hall, 1987; Wong *et al.*, 1992). Sowing seed naturally infected with *F. graminearum* resulted in reduced seed germination and stand density in the field (Duthie and Hall, 1987). They found as the incidence of infected seed sown was increased so the number of infected shoots and tillers increased.

1.4 Epidemiology

1.4.1 Sources of inoculum

Figure 1.1 shows that *Fusarium* inoculum can come from a number of sources including, crop debris at the base of the crop and plants suffering with foot rot or seedling blight. All the main *Fusarium* pathogens and *M. nivale* can survive saprophytically on straw debris (Parry *et al.*, 1994). Sutton (1982) suggested that stubble of wheat and other cereals, as well as stalks and ears of maize were the main sources of inoculum of *F. graminearum*. Clear and Abramson (1986) commented that the incidence of FEB was significantly greater if maize was grown within two years prior to a crop of wheat. Similarly, the incidence of *F. culmorum* propagules in soil was far greater within continuously grown wheat, than from fields sown continuously under broad leaved crops (Snyder and Nash, 1968).

It is suggested that Fusarium foot rot in the developing wheat crop is one of the important sources of inoculum for FEB (Figure 1.1). In a national survey on the incidence and severity of FEB in UK winter wheat crops between 1976 and 1988, over 80 % of crops surveyed had foot rot (Polley and Thomas, 1991). A specific survey of *Fusarium* species in stem base diseases of winter wheat in the Midlands of the UK between 1987 and 1989 found that *M. nivale* accounted for up to 65 % of infections and *F. avenaceum* up to 60 % (Parry, 1990). These studies indicate that Fusarium foot rot is a relatively common disease in temperate regions which would provide a consistent source of inoculum for development of FEB.

Alternative hosts can also provide an important source of inoculum. In Canada, Gordon

(1959) isolated *Fusarium* spp. from 173 plant species, including 19 grass species (including cereals), 19 vegetable species, 15 fruit crops, 52 ornamental plants, 31 trees and shrubs, 24 weed species and 13 other field crops. In the UK, Jenkinson and Parry (1994a) obtained 226 *Fusarium* isolates from 14 weed species, including plants within the Compositae, Ranunculaceae, Polygonacae, Cruciferae and Chenopodiacae, despite no obvious symptoms of *Fusarium* infection being observed on these plants. Seventy five of these isolates, from a total of 77, including *F. avenaceum, F. culmorum, F. graminearum* and *F. poae* were found to be pathogenic on seedlings of winter wheat. The importance of this potential reservoir of inoculum is, however, unclear, but it does suggest that weed control may prove an effective way of reducing the level of inoculum.

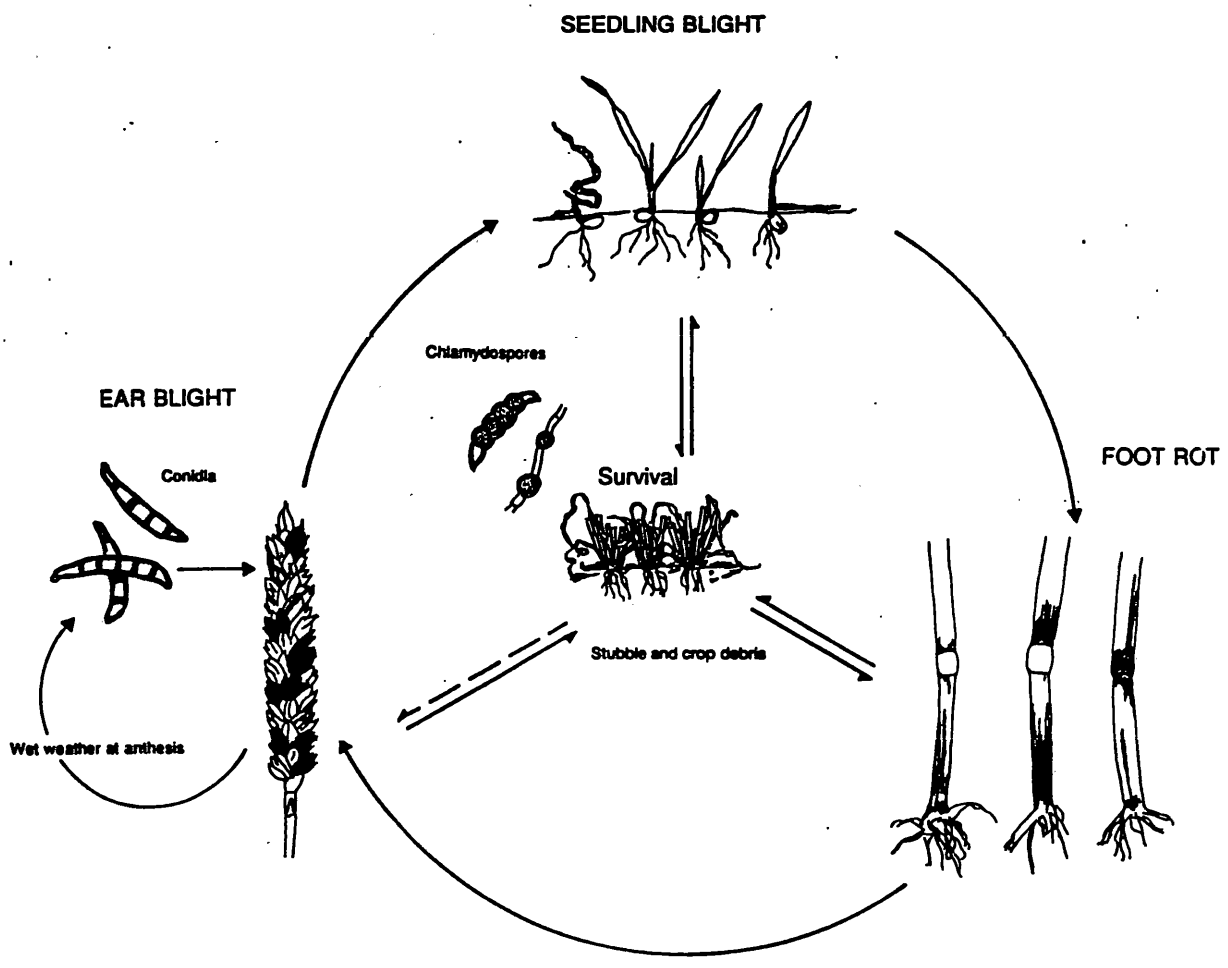


Figure 1. Generalised disease cycle of *Fusarium* on small grain cereals (from Parry *et al.*,1994).

1.4.2 Dispersal of inoculum

Dispersal of inoculum from the base of the crop to the ears is a matter of some debate. Some workers have suggested that the pathogen may grow systemically through the stem from the stem base to the ear. For example, Jordan and Fielding (1988) observed systemic infection of ears following artificial inoculation of wheat seedlings below soil level with *F. culmorum* and then re-isolating the pathogen from all internodes as well as the ear in some plants. Similarly, Hutcheon and Jordan (1992) were able to show systemic infection of wheat with *F. avenaceum, F. graminearum, F. culmorum* and *M. nivale* by placing a mycelial plug just below the soil so that they were in contact with a plant at GS 21 (Zadoks *et al.*, 1974). To prevent infection by external inoculum, plants were placed in clear plastic bags and growth of each species was shown by re-isolating the fungus from internode tissue and in some cases from the ear.

In contrast, conflicting evidence is available to suggest that FEB is not caused by systemic infection. For example, Atanasoff (1920) inoculated individual seeds of winter wheat with conidia of *F. graminearum* and managed to obtain symptoms of foot rot, but no fungus was re-isolated from the ear. Snijders (1990b), found evidence of infection as high as 70 cm above soil level, but failed to re-isolate *F. culmorum* from ears of wheat following soil inoculation. However, it is now believed that systemic infection is not important in infecting wheat ears (Clements and Parry, 1998). A recent study using scanning electron microscopy showed that there was no evidence of systemic infection above the fourth node when wheat seedlings were sown into soil infected with *F. graminearum, F. culmorum* and *M. nivale* (Clements and Parry, 1998). Although there was evidence of upward growth from the stem base by all species, most of this

growth was shown not to be systemic growth but rather colonisation of protected exterior plant surfaces.

Some researchers are of the opinion that *Fusarium* infection of the ear is caused by wind-blown or rain-splashed spores infecting wheat ears. In Ontario, Sutton (1982) related epidemics of FEB to above average rainfall during July in five out of six severe years between 1927 and 1980. In the Netherlands, a significant correlation between percentage infected spikelets showing symptoms of FEB and total rainfall during the period 11 June - 11 July was observed for the years 1979 - 1986 (Snijders, 1990c).

It is possible that rain or heavy dew is required for the release of macroconidia from sporodochia and ascospores from perithecia. When drops of water were placed onto sporodochia of *F. graminearum*, spores were readily released (Atanasoff, 1920). Using a rotorod spore trap in a plot of winter wheat, Millar and Colhourn (1969) observed that during periods of dry weather, few spores were caught. Following rain the concentration of spores (conidia and ascospores) increased between 10 and 20 times peaking 10 minutes after rain had stopped. Although ascospores were relatively common 15 cm above ground, none were trapped at ear height. In Europe, many outbreaks of FEB have been attributed to the production of ascospores by *F. graminearum*. Suty and Muler-Machnik (1996) after measuring the daily dispersal of ascospores at a number of sites in Germany found that increases in ascospores trapped coincided with rain shortly before or during the day of spore ejection. A more detailed study on ascospore release used plots inoculated with *G. zeae* colonised maize during May before spore counts were made every 15 minutes using a spore sampler (Paulitz, 1996). This study revealed

that the daily release of ascospores by *G. zeae,* coincided with an increase in humidity around 1600 to 1800 hours, reached a peak at midnight, and declined to low levels by 0900.

The relationship between heavy rainfall at anthesis and infection of wheat ears by *Fusarium* spp is unclear. It is possible that spores released following heavy rainfall can be dispersed to the wheat ear in splashed droplets of rain. Similarly, the number of conidia of *M. nivale, F. culmorum* and *F. avenaceum* caught in a spore trap during June 1992 was closely related to the amount of rainfall; in particular heavy rain storms were characterised by massive increases in the number of conidia trapped (Jenkinson, 1994). Jenkinson and Parry (1994b) measured how far conidia of *F. culmorum* and *F. avenaceum* could be dispersed when incident water drops, 5 mm in diameter and travelling at terminal velocity, were allowed to fall onto stem bases of wheat bearing sporodochia. Conidia were caught on strips of selective media (100 x 3 cm) set out at various distances from the inoculum. Colonies of *F. culmorum* and *F. avenaceum* were observed at maximum heights of 60 and 45 cm, respectively, and at maximum horizontal distances of greater than100 and 90 cm. Although this showed the vertical movement of spores in droplets of water it still did not reveal how spores reached the ear. However, Jenkinson and Parry (1994b) suggested that spores could be carried to the ear in a series of 'leaps' involving the infection of the upper plant parts, similar to that seen with *Septoria tritici* (Royle *et al.*, 1986; Lovell *et al.*, 1997).

1.4.3 Conditions required for disease development

Favourable weather is critical for the successful infection of wheat ears by *Fusarium*

spp. Atanasoff (1920) was the first to observe the period of time that elapsed before the first symptoms appeared in a field experiment artificially inoculated with *F. graminearum*. In wet weather, this time varied from 3 to 6 days, whilst in dry weather, symptoms did not appear until the first rain or heavy dew. Anderson (1948) showed that temperature also influences infection following artificial inoculation of wheat with *F. graminearum*. Infection was most successful at 25°C, following 72 h of continuous wetness, under a humidity tent, causing 96 % spikelet infection, whilst at 20 and 30°C only 81 % and 86 % were observed, respectively. After 48 h under continuous wetness, infection was again greatest at 25°C causing 77 % spikelet infection, but greatly reduced at 20°C and 30°C causing only 5 % and 27 %, respectively. More recently, Lacey (1989) suggested that infection by *Fusarium* spp is favoured by temperatures of 20-30°C with surface wetness (dew) persisting for 48-60h, whilst temperatures of <15°C or surface wetness for <24h prevented infection. The importance of high humidity for successful infection under field conditions was shown by Jennings and Turner (1996). Following inoculation with individual isolates of either *F. avenaceum, F. culmorum, F. graminearum, F. poae* or *M. nivale,* wheat plots were subjected to two humidity regimes. These were high: where relative humidity at ear height was maintained at 80 % using mist irrigation and ambient conditions. These workers found that increasing humidity produced earlier development and increased incidence of disease for all species except *F. poae*. In Argentina, attempts have been made to predict the incidence of FEB using models based on meterological conditions (Moschini and Fortugno, 1996). These workers found strong associations between the incidence of FEB and the number of two day periods with rainfall and humidity >81 % in the first day, and relative humidity ≥78 % in the second.

More detailed studies of the effects of water activity (a_w) and temperature on growth of *F. culmorum, F. avenaceum, F. poae* and *F. tricinctum* have been studied *in-vitro* using wheat extract media modified with glycerol (Magan and Lacey, 1984). The optimum temperature for growth in all species at 0.995 a_w was 25°C. The optimium and minimum a_w for growth of *F. culmorum, F. avenaceum* and *F. tricinctum* was 0.995-0.99 and 0.89-0.90 at 25°C, whilst for *F. poae* the optimum a_w decreased from 0.995-0.98 as temperature increased from 25 to 30°C (Magan and Lacey, 1984).

1.5 Control

1.5.1 Cultural control

Cultural methods for the control of FEB involve either eliminating sources of inoculum or creating conditions that are not conducive to disease development. One method to reduce the amount of inoculum is to remove straw prior to sowing. In New South Wales, Australia, burning of straw was effective in maintaining a low incidence of FEB over four years compared with straw retention (Burgess *et al.*, 1996). In the European Community the practice of straw burning has been banned since 1992 and as a result, ploughing in of crop debris is the main approach adopted towards stubble hygiene. Wilcoxson *et al.* (1988) have shown that incorporation of stubble by ploughing can reduce the incidence of FEB in wheat crops compared to when debris is left on the soil surface.

By using certain crop rotations, a grower can reduce the amount of inoculum that can infect the subsequent crop. For example, maize is another host for *Fusarium* spp and therefore FEB is more severe in wheat crops which follow maize in the rotation. Teich

and Nelson (1984) found that the average incidence of FEB in crops of wheat which followed maize was six to seven times greater than in wheat crops which followed soybeans or cereals (wheat, barley, oats).

1.5.2 Chemical control

Control of FEB on wheat with fungicides under field conditions has proved inconsistent due to poor ingredients (Parry *et al.*, 1995), lack of knowledge about application timing (Hutcheon and Jordan, 1992) and the detrimental effect of these compounds on beneficial micro-flora that reduce levels of *Fusarium* infection on wheat ears (Liggitt *et al.*, 1997). Milus and Parsons (1994) reported that in a field trial artificially inoculated with *F. graminearum,* there was no reduction in FEB incidence following the application of a number of fungicides (benomyl, chlorothalonil, fenbuconazole, flusilazole, myclobutanil, potassium bicarbonate, propiconazole, tebuconazole, thiabendazole and triadimefon plus mancozeb). However, some active ingredients have shown limited control of FEB. For example, prochloraz was shown to give significantly better control of FEB in a glasshouse trial artificially inoculated with *F. culmorum* compared with eight other fungicides (Hutcheon and Jordan, 1992). Tebuconazole has also shown effective reduction of FEB in field trials in Hungary (Mesterhazy and Bartok, 1996), France and Germany (Suty and Muler-Machnik, 1996). More recently, products containing stobilurins have shown good control of a range of pathogens (Godwin *et al.*, 1992). Although such compounds have shown good control of *M. nivale* in the field they have not proved effective against *F. culmorum* (J.Froggett. personal communication).

Another form of chemical control could include the use of growth regulators to prevent lodging by reducing the height of the wheat crop. For example, a field study in Norway on the effect of lodging in barley and oats showed that DON levels were nearly twice as high in lodged plots compared to those which remained standing (Langseth and Stabbetorp, 1996). These authors assumed that the increase in DON in lodged plots was due to *Fusarium* infection although they presented no data to confirm this. However, the growth regulators ethephon and chlormequat have been shown to increase the severity of FEB during a field trial of wheat inoculated with *Fusarium*-infected maize kernels (Fauzi and Paulitz, 1994). They believed that the shortened plants were subject to higher inoculum doses because they were closer to ejected ascospores from infected stem bases and stubble.

1.6 Cultivar resistance

Since control of FEB through the use of fungicides has proved so inconsistent, the breeding of cultivars resistant to FEB is therefore important component in controlling of the disease.

1.6.1 Sources of resistance

Arthur (1891) was the first to observe differences in FEB susceptibility between cultivars of wheat, noting that early maturing cultivars tended to be more resistant than later ones. The importance of resistant cultivars of wheat was acknowledged by Dickson and Mains (1929) who reported that the FEB susceptible cultivar, Progress, was being less widely grown in Illinois, than the FEB resistant cultivar Illinois no.1 because of yield losses associated with the disease. Atanasoff (1924) also noted differences in the

susceptibility of 30 cultivars of spring and winter wheat during naturally infected field experiments. A more extensive survey of 350 spring cultivars and hybrids of *Triticum* spp. showed differences in susceptibility with all lines showing some symptoms following inoculation with a mixture of *Fusarium* species (Christensen *et al.*, 1929). Hanson *et al.* (1950) also found differences in susceptibility when several hundred cultivars and several thousand hybrids were tested under naturally infected and artificially created epidemic conditions. Snijders (1990a) during four years of field experiments also found large variation in resistance among a total of 258 winter and spring wheat genotypes after inoculation with *F. culmorum.* Although a number of Eastern European winter wheat cultivars were found to be resistant (less than 10 % of spikelets showing symptoms of FEB) a higher proportion of spring cultivars were found to be resistant. It is possible that as spring wheat cultivars mature earlier, flowering may not coincide with high levels of inoculum or a more favourable climate for infection (Cook, 1981) as with winter cultivars.

Screening for resistance to FEB is now conducted in most major wheat growing countries worldwide in either research institutions or by commercial breeding companies. Table 1.1 summarises some of the more recent screening programmes.

To date, the more important sources of resistance discussed in the literature include the spring wheats Sumai 3 from China (Liu and Wang, 1991), Frontana from Brazil (van Ginkel *et al.*, 1996) and Nobeoka-bozu from Japan (Mesterhazy, 1995)(Plate 1.1).

(a) (b)

Plate 1.2 Field plots of the resistant cultivars (a) Sumai-3 and (b) Nobeoka bozu at the Cereals Research Institute, Szged, Hungary, May 1996 articially inoculated with *Fusarium* spp.

Table 1.1 Some recent international screening programmes for resistance to FEB (after Parry *et al.*, 1995).

Country	Type of inoculum	Notes	Reference
Argentina	Artificial inoculation with *F. graminearum* in the glasshouse and the field	Breeding programme to develop genotypes with improved resistance to FEB. To date, about 20 lines have shown similar resistance to Sumai 3 under test conditions.	de Galich (1997)
Austria	Artificial inoculation with a mixture of *F. graminearum* and *F. culmorum*	Screening of 96 winter wheat genotypes and 38 spring wheat genotypes.	Buerstmayr *et al.* (1996)
China	Artificial inoculation with *F. graminearum*	Screening of >30, 000 wheat genotypes over 9 years. Pyramiding of FEB resistance from moderately susceptible Italien and Chinese cultivars resulted in enhanced resistance.	Liu and Wang (1991)
Canada	Natural infection	Survey of wheat cultivars grown in Ontario. Cultivars differed in both FEB incidence and DON concentration in grain.	Teich *et al.* (1987)
France	Inoculated with *F. culmorum*	Screening of 564 genotypes from 15 *Triticum* spp.	Saur (1991)
Germany	Artificial inoculation with *F. culmorum*	Screening of 59 wheat genotypes over 2 years.	Miedaner and Walther (1987)
Hungary	Artificial inoculation with *F. graminearum* and *F. culmorum* and natural infection	Screening of 25 wheat genotypes showed that although cultivars were similar in FEB severity they were significantly different in yield, suggesting tolerance to infection in some genotypes.	Mesterhazy (1995)
India	Artificial inoculation with *F. graminearum*	Screening of 127 Indian genotypes	Brahma (1988)

Country	Type of inoculum	Notes	Reference
Japan	Artificial inoculation with *F. graminearum*	Genetic analysis of FEB resistance using 899 double haploid (DH) and recombinant inbred lines. Four random amplified polymorphic DNA (RAPD) markers were associated with resistance in some DH lines.	Ban (1997)
Mexico	Artificial inoculation	Screening of 7,000 lines from traditional breeding and wide crosses at CIMMYT	Gilchrist *et al.* (1997)
Netherlands	Artificial inoculation with *F. culmorum*	Screening of 258 winter and spring wheat genotypes.	Snijders (1990a)
Romania	Artificial inoculation with *Fusarium* spp	Screening of 108 genotypes of wheat.	Mariana *et al.* (1997)
Russia	Artificial inoculation and natural infection	Annual screening of 1500 winter selections and 200 local and foreign wheats.	Ablova and Slusarenko, (1997)
UK	Artificial inoculation with *F. culmorum* and natural infection	Screening of 19 cultivars using natural infection and 58 cultivars following artificial inoculation.	Parry *et al.* (1984)
Uruguay	Artificial inoculation with *F. graminearum*	Research to identify resistance among local, Chinese and Mexican genotypes.	Diaz de Ackermann and Kohli (1997)
USA	Artificial inoculation with *F. graminearum*	Breeding programme to produce cultivars with improved resistance. Involves screening 36 genotypes at six different locations.	Rudd (1997)
former Yugoslavia	Artificial inoculation with *F. graminearum*	Screening of 870 genotypes from all over the world. Improved resistance in genotypes obtained from crosses.	Koric and Tomasovic (1991)

Within the genus *Triticum* there are differences between species in susceptibility to FEB. Christensen (1929) and later Hanson *et al.* (1950) showed that, in general, durum

wheats were more susceptible to FEB than common wheat, although considerable variation in resistance existed among the durum wheats. Similarly, significant variation has been found in susceptibility to FEB in triticale. For example, 34 triticale genotypes at three sites were artificially inoculated with *F. graminearum* and sufficient variation was demonstrated to select for superior genotypes (Dormann and Oettler, 1993).

Other species related to wheat have also shown a high degree of variation to FEB resistance. Extensive work is underway in Germany (Miedaner *et al.*, 1995) towards breeding cultivars of winter rye resistant to FEB. Single plants from the self incompatible, open pollinated winter rye cultivar Halo, representing the 'Petkus' gene pool, were crossed pairwise to obtain full-sib families. Artificial inoculation with *F. graminearum* of two sets of 93 full-sibs revealed ample genetic variation for resistance to FEB in winter rye. Valuable sources of resistance have also been found amongst lines of *Hordeum chilense* (wild barley) and *H. chilense x Triticum* spp amphiploids named triodeums (Rubiales *et al.*, 1996). They believed that ergosterol analysis, used as a measure of fungal biomass following artificial inoculation with *F. culmorum,* showed that resistance to colonization was highest for *H. chilense* followed by tritodeum and wheat. However, as ergosterol is a lipid found in the cell membranes of most fungal species it is possible this analysis was detecting colonisation by other fungal species as well as *F. culmorum*. In India, Brahma (1988) found that six out of nine *Agropyron* species, artificially inoculated with *F. graminearum* in the field were immune to FEB whilst the other three showed moderate resistance. This is the only report of immunity to FEB although there may be resistant material in other related species.

Including resistance to visual symptoms there is also evidence of tolerance to infection by *Fusarium* spp in some cultivars of wheat (Mesterhazy, 1995). Tolerance can be defined as the abiltiy of a cultivar to endure infection by a pathogen without a significant yield loss that could occur in an equivalent cultivar. For example, Mesterhazy (1995) found that among 25 genotypes in a field experiment, five showed no significant difference in FEB severity, but differed significantly in yield after artificial inoculation with *F. graminearum* and *F. culmorum*. Although tolerant cultivars would have reduced yield loss caused by FEB grain could still be affected by mycotoxin contamination.

1.6.2 Screening for resistance

Various methods have been used to establish FEB epidemics in order to screen cultivars for resistance. Scott (1927) found that naturally-infected field experiments in Minnesota, USA, failed to give sufficient infection to compare cultivars. In contrast, Wiersma *et al.* (1996) found suitable levels of FEB incidence and severity in Wisconsin, USA, relying on natural infection. However, Liu and Wang (1991) found that natural epidemics of FEB only occurred once every 4 years in China which was not considered reliable enough to assess cultivar resistance. Due to the unreliable nature of natural infection in the field, the majority of screening programmes throughout the world have relied upon artificial inoculation (Mesterhazy, 1995; Snijders 1990a; Parry *et al.*, 1984). Climatic conditions, in particular humidity, may not always favour infection, so many workers have used clear polythene bags placed over inoculated ears (Mesterhazy, 1995) or overhead mist irrigation to ensure high humidity for epidemics to develop in wheat (Wilcoxson *et al.*, 1992) and in winter rye (Miedaner *et al.*, 1993).

The choice of disease assessment method depends on the aim of the study and the availability of resources. Individual necrotic spikelets have been counted where spread of the disease has been studied following inoculation (Bai and Shaner, 1996a) or where an accurate scoring method is required (van Ginkel *et al.*, 1996). In a screening programme, this method may be time consuming, hence disease keys such as those documented by Parry *et al.* (1984) and Snijders (1990a) have been devised.

The assessment of FEB relies on the use of mature plants which need up to 9 months to grow. Thus many workers have attempted to reduce this time by relating symptoms of seedling blight or foot rot as a means of predicting the response of FEB on the mature plants (Mesterhazy, 1987; Arsenuik *et al.*, 1993; Maurin *et al.*, 1996). In order to assess seedling blight, Mesterhazy (1984) chose to use height and dry matter production of infected seedlings, whilst Wildermuth (1994) and Arsenuik *et al.* (1993) used a 0-4 scale based on seedling symptoms. Significant relationships between symptoms of seedling blight and severity of FEB resistance have been observed among genotypes of winter wheat following inoculation with a range of *F. culmorum* and *F. graminearum* isolates (Mesterhazy, 1984; 1987) and with *F. graminearum* (Wildermuth, 1994). In contrast, no significant correlations were found between seedling and FEB reactions with *Fusarium* spp. on 17 triticale cultivars and two rye cultivars (Arseniuk *et al.*, 1993). As there is no direct relationship between symptoms of seedling blight and FEB, it is not considered a reliable way to identify genotypes with an intermediate level of resistance (Wildermuth, 1994). Maurin *et al.* (1996) measured the stem lesion area to assess susceptibility to stem rot caused by *M. nivale* following inoculation with an agar disk placed at the base of wheat seedlings at the three leaf stage (GS 13). When the data

from 33 genotypes of wheat was correlated with FEB severity in the field no significant association was observed.

1.6.3 Nature of resistance

Resistance of wheat to FEB pathogens is believed to share a similar genetic background. Scott and Benedikz (1986) observed in a field trial in the UK, that a number of test cultivars were ranked similarly for resistance following artificial inoculation with separate isolates of *F. graminearum, F. culmorum, F. avenaceum* and *F. poae*. This data contradicted earlier reports which claimed that there were three physiological forms of *F. graminearum* and *F. culmorum* and two forms of *F. avenaceum* which were differentiated by their response on different cultivars of wheat (Tu, 1930). Most workers believe that resistance to FEB in wheat is not species-dependent and is controlled by race non-specific genes. The most extensive study on this subject was performed over three years at six locations across Europe by van Eeuwijk *et al.* (1995). Although only one strain of *F. culmorum* was used to inoculate 25 genotypes of winter wheat at all sites, ten strains of *F. culmorum,* six strains of *F. graminearum* and one of *M. nivale* were used at individual sites to create 59 year by strain by location combinations. The results showed that resistance to these pathogens was non-specific. Non-specific resistance has also observed in rye when 16 inbred lines were inoculated with *F. graminearum* and *F. culmorum* and found to be closely related (r = 0.96-0.97) for disease rating and yield (Miedaner *et al.*, 1993).

Within *F. culmorum,* Snijders (1987) claimed that he had identified seven isolates of the pathogen which showed specific interactions when 18 genotypes of wheat were

inoculated with 18 isolates. He then grouped these 18 isolates in terms of horizontal and vertical races (Vanderplank, 1984). However, more recent work could find no evidence to support race-specific resistance when 17 genotypes of winter wheat were inoculated with four individual isolates of *F. culmorum* in a three year field experiment (Snijders and van Eeuwijk, 1991). It was concluded, therefore, that resistance to *F. culmorum* could be explained in terms of horizontal resistance as defined by Vanderplank (1984).

Although isolates of *Fusarium* spp. do not show particular host specificity they do differ in pathogenicity. Arsenuik *et al.* (1993) showed that pathogenicity of 20 isolates of 12 *Fusarium* species, recovered from triticale seed, differed significantly on seedlings of wheat, triticale and rye with the following species being individually the most pathogenic, *F. avenaceum, F. culmorum, F. graminearum* and *F. sambucinum* var. *coeruleum*. However, the most pathogenic inoculum was a mixture of isolates of all the species which produced similar ranking of cultivars when compared with single isolates (Arsenuik *et al.*, 1993). It can therefore be concluded that using mixtures of isolates is a sensible way to assess resistance to FEB in wheat.

A number of studies on the inheritance of FEB resistance in wheat have suggested that it is a quantitative character controlled by a number of genes. For example, Nakagawa (1955) studied the segregation ratios of healthy to infected grains from F_2 plants from seven crosses of Japanese cultivars. Data from this study suggested that three genes were involved in FEB resistance. Bai *et al.* (1989), who after carrying out a diallel cross between three resistant and three susceptible cultivars of winter wheat, concluded that there were three major resistance genes showing partial or full dominance which were

modified by a number of minor genes. Analysis of F_1 and F_2 generations from crosses made from ten genotypes representing different resistance to FEB indicated that the number of segregating genes varied between one and six (Snijders, 1990d). One technique that can be used to locate genes on specific chromosomes is monosomic analysis, where a number of lines are produced without individual chromosome pairs. Using this technique to study the inheritance of resistance in Sumai 3 X Chinese spring F_2 populations, Yu (1982) found reduced spikelet infection when any one of chromosome 1B, 2A, 5A, 6D or 7D was missing.

Inheritance studies have been performed using the resistant spring wheat cultivar Frontana. Singh *et al.* (1995) evaluated 337 random F_6 lines derived from crosses of Frontana with susceptible, or moderately susceptible cultivars following inoculation with a mixture of *F. graminearum* isolates. Quantitative and qualitative models indicated that resistance of Frontana was controlled by the additive interaction of at least three minor genes. Transgressive segregation towards resistance indicated that some of the susceptible lines also carried one or two minor genes. In a separate study by van Ginkel *et al.* (1996), Frontana was compared with the highly resistant Chinese cultivar Ning 7840. Random F_2 -derived F_7 lines from six crosses between the resistant and susceptible lines were artificially inoculated with *F. graminearum* and symptom spread was measured. Diallel analysis, according to Wright (1968), indicated that the two resistant parents each possessed two unique dominant genes which could be combined to give higher levels of resistance.

As well as recognising resistant parents, it is important to know whether individual

parents have good combining ability to produce resistant progeny. Two types of combining ability exist, general combining ability (GCA) which refers to the ability of an individual parent to produce progeny of a given performance when mated with a number of other parents, and, specific combining ability (SCA) which refers to the performance of a specific parental combination. For example, a cross between two parents may result in progeny that are more resistant than the original parents, whilst another cross with one of the parents with a third parent may result in offspring that are less resistant. Snijders, (1990e) assessed combing ability in ten winter wheat genotypes by performing a half diallel cross (excluding reciprocals) and then analysing the data according to Griffing (1956). This experiment showed significant GCA effects for both F_1 and F_2 but no significant SCA following inoculation with *F. graminearum*. Estimates of combining ability from 40 single hybrid crosses of winter rye indicated that GCA was ten to six times larger than SCA over 2 years (Miedaner and Geiger, 1996).

As the majority of wheat lines possessing resistance to FEB have high GCA they should produce resistant progeny when crossed with a series of other parents. Snijders (1990f) followed a 10 x 10 half diallel and estimated that resistant parents differed in one or two resistance genes. He believed that these could be accumulated to produce a more resistant line. Liu and Wang (1991) reported that by combining moderately susceptible Italian cultivars with moderately susceptible Chinese cultivars they were able to enhance FEB resistance.

1.7 Aims of the project

Although good sources of resistance to FEB have been identified, (Parry *et al.*, 1984; Snijders 1990a; Mesterhazy, 1995) documented literature on the specific mechanisms controlling resistance is limited. The first aim was to identify factors which could explain differences between wheat cultivars in resistance to initial infection and colonisation by *Fusarium* spp. Resistance to infection was studied using *in-vitro* tests developed to assess the effect of anthers from different cultivars on the initial growth of conidia of *F. culmorum*. Resistance to colonisation was studied in different cultivars by point inoculating a central spikelet with *F. culmorum* or *F. graminearum* and then assessing symptom development and fungal infection. It is hoped that a greater understanding of the mechanisms of resistance to FEB will lead to more efficient procedures to identify and screen suitable genotypes.

Although a number of wheat genotypes with good resistance to FEB have been identified, most have agronomically unsuitable characteristics. A second aim of this work was therefore to reveal other morphological characters that could be related to resistance. Differences in straw height, peduncle length, compactness of ear and total leaf area were assessed between cultivars and related to severity of FEB in artificially inoculated field plots. Recent evidence suggests that there is a link between cultivar height and severity of FEB, with shorter cultivars showing more severe symptoms (Mesterhazy, 1995). To determine if the relationship between straw height and severity of FEB was caused by a genetic association or an effect of the microclimate, height and disease were measured in segregating populations derived from tall X short cultivars and humidity was measured in near-isogenic lines with and without the *Rht1* and *Rht2*

dwarfing genes. It is hoped that such work will assist in the production of genotypes with agronomically good characters including resistance to FEB.

CHAPTER 2

General materials and methods

2.1 Culture of host

2.1.1 Production of plant material

Seed from a number of cultivars of wheat differing in their resistance to FEB in the field (Table 2.1) were sown into separate seed trays (20 x 15 x 4.5 cm) containing John Innes N° 1 compost at a rate of 200 seeds per tray. In order to allow germination, seed trays were then placed in a glasshouse at 20 ± 3°C for 7 days. Fully emerged seedlings were then placed in an artificially illuminated refrigerator set at 4°C with an 8 hour photoperiod for 8 weeks to allow vernalisation. For each cultivar, five vernalised seedlings were then transplanted into plastic pots (15cm in diameter) containing John Innes N°1 compost and placed in a glasshouse set at 20 C +/º 3 C and with a photoperiod of 12 hours.

The base of each pot was watered daily and fed once a week with an application of the foliar fertiliser 'Phostrogen'(10 % N, 10 % P_2O_5, 27 % K_2O) (Phostrogen Ltd, Corwen, Clwyd, UK). When required, plants were sprayed with 'Aphox' (primicarb 0.9g/l water; Zeneca) to control aphids and 'Corbel' (fenpropimorph 2.5g/l water; BASF) to control powdery mildew.

Table 2.1 Cultivars of wheat used in glasshouse experiments during this study.

Cultivar	Date first listed on NIAB list	Reference	NIAB rating	Pedigree / Breeder
Admiral	1992	Anon (1995)	5	Mithras X Hobbit (ICI seeds, UK)
Apollo	1988	Anon (1989)	6	Maris Beacon X Kronjuwel (Saatzucht Joseph Breun, Germany)
Avalon	1980	Parry *et al.* (1984)	4	TJB 30/148 X TL 365a/34 (Plant Breeding International, UK)
Beaver	1990	Anon (1995)	6	(Hedgehog X Norman) X Moulin (Plant Breeding International, UK)
Brigadier	1993	Anon (1995)	6	Squadron X Rendezvous (ICI seeds, UK)
Genesis	1993	Anon (1995)	6	Arminda X TJB 363 (Serasem, France)
Haven	1990	Anon (1995)	5	(Hedgehog X Norman) X Moulin (Plant Breeding International, UK)
Hereward	1991	Anon (1995)	6	Norman "sib" X Disponent (Plant Breeding International, UK)
Hunter	1993	Anon (1995)	5	Apostle X Haven (Plant Breeding International, UK)
Hussar	1992	Anon (1995)	5	Squadron X Rendezvous (ICI seeds, UK)
Kraka	-	Buerstmayr *et al.* (1996)	-	-
Mercia	1986	Anon (1995)	5	(Talent X Virtue) X Flanders (Plant Breeding International, UK)
Riband	1989	Anon (1995)	6	Norman X (Maris Huntsman X TW 161) (Plant Breeding International, UK)

Cultivar	Date first listed on NIAB list	Reference	NIAB rating	Pedigree / Breeder
Sumai -3	-	Liu and Wang (1991)	-	-
Virtue	-	Parry *et al.* (1984)	-	-

2.2 Culture of pathogen

2.2.1 Aseptic techniques

Culturing of pathogens, isolating *Fusarium* from infected plant material and pouring of media into plastic 9 cm Petri dishes (Bibby sterilin Ltd, Stone, Staffordshire, UK) was performed in a sterile laminar flow cabinet. Equipment used in sterile procedures including glassware, media and distilled water was sterilised by autoclaving at 121°C and 103.4 KPa for 20 minutes.

2.2.2 Preparation of isolates

Pathogenic isolates of *F. culmorum, F. avenaceum, F. graminearum* and *M. nivale* that had been used in field experiments at Harper Adams and Plant Breeding International (Table 2.2) were studied in this work. Isolates were maintained by taking mycelial plugs, 5mm in diameter, from the edge of an actively growing culture and placing them into separate plates of potato dextrose agar (PDA) (Unipath Ltd, Basingstoke, UK) amended with streptomycin sulphate (100 mg/l) and chlorophanicol (50mg/l) (Sigma Chemical Ltd, Dorset, UK). All plates were then sealed with 'Parafilm' tape (Nescofilm, Nippon Shoju Kaisha Ltd, Osaka, Japan) and incubated in darkness at 20°C for 14 d. To store cultures, plates were sealed and kept in a refrigerator set at 4°C. For long-term

storage, cultures were stored as spore suspensions in glass ampoules and stored under liquid nitrogen. To maintain culture viability spore suspensions of individual isolates were made and applied to detached wheat ears that had been surface sterilised by placing in 5 % sodium hypochlorite solution (5 % available chlorine) for 20 seconds before being rinsed in two changes of sterile distilled water. The ears were then set on moist tisuue paper in a Petri dish and placed in a dark incubater set at 20°C for 1 week to allow symptoms to develop. Individual spikelets were then detached and placed onto Petri dishes containing fresh PDA and incubated in darkness at 20°C till colonies grew enough to be re-isolated onto fresh PDA.

Table 2.2. Details of isolates of *Fusarium* spp and *M. nivale* used in this study.

Isolate code	Isolate species	Host of origin	Place of origin
F 94-6	*F. avenaceum*	Wheat	Plant Breeding International (Cambridge)
F 90-2	*F. culmorum*	Wheat	Plant Breeding International (Cambridge)
F 94-8	*F. graminearum*	Wheat	Plant Breeding International (Cambridge)
F 91-3	*M. nivale*	Wheat	Plant Breeding International (Cambridge)
416/10	*F. culmorum*	Wheat	Harper Adams Agricultural College

2.2.3 Preparation of inoculum

In order to obtain conidia, 14 day old cultures were placed in an incubator under near ultra violet light (350μW/cm^2) at 20°C for 3 weeks until sporodochia were observed on the surface of mycelium. For each isolate, a conidial suspension was prepared by

flooding colonies with sterile distilled water and dislodging conidia with the aid of a sterile spatula. The conidial suspension was then filtered through two layers of muslin to remove hyphal fragments before determining the spore suspensions using a haemocytometer (Webster Scientific International Ltd, UK). For field trials, inoculum of known concentration was poured into clear plastic bags, stored in a freezer set at -20°C and thawed when needed for inoculation.

CHAPTER 3

Factors affecting resistance to initial infection by *Fusarium culmorum* in winter wheat.

3.1 Introduction

It is generally believed that initial infection of wheat ears by *Fusarium* species occurs during anthesis. Arthur (1891) was first to observe that FEB was associated with infection of the wheat flower. Atanasoff (1920) showed that when ears of wheat in anthesis (GS 65) were artificially inoculated with *F. graminearum* they were more susceptible to infection than ears of wheat inoculated at ear emergence (GS 59) and late dough stage (GS 87) (Zadoks *et al.*, 1974). Similarly, Anderson (1948) observed no infection on ears inoculated with *F. graminearum* before flowering but found that ears became more susceptible as anthesis proceeded, so that those ears inoculated after flowering showed 100 % spikelet infection. A similar study revealed that when the anthers were removed from wheat ears (emasculated) and inoculated with *F. graminearum,* most ears remained healthy compared with non-emasculated ears (Strange and Smith, 1971).

Although this evidence shows the importance of anthesis to infection by *Fusarium* spp., it does not show anthers to be the site of infection. Indeed, Tu (1930) suggested that infection of wheat ears by *F. graminearum* was via the glumes. Using a small drop of inoculum to inoculate the outer glumes of wheat plants, successful infection was obtained from ear emergence (GS 59) until the soft dough stage (GS 85). However, histological studies carried out by Pugh *et al.* (1933) revealed that cross sections of a diseased spikelet infected with *F. graminearum,* showed no evidence of the pathogen growing on the outside of glumes although there was substantial fungal growth around the anthers.

Further, indirect evidence, for anthers being the initial site of infection comes from results that suggest these structures contain fungal growth stimulants. Using mass spectrometry, stimulatory compounds in anthers have been identified as choline chloride, betaine hydrochloride with a third unidentified compound (Strange *et al.*, 1974). These compounds, although found in glumes and grain were found in far higher concentrations in anthers (Pearce *et al.*, 1976). Strange *et al.*(1974) showed that when pieces of filter paper soaked with either anther extract, betaine or choline were applied to florets of winter wheat inoculated with *F. graminearum,* increased infection occurred. However, use of filter papers soaked with sucrose was no more stimulatory than water at encouraging infection. Despite this, Strange *et al.*(1974) believed that choline and betaine were essential nutrients required to enhance virulence in *Fusarium* spp. *In-vitro* tests showed that choline and betaine promoted hyphal extension of both *F. avenaceum* and *F. culmorum* but not of *M. nivale* (Strange and Smith, 1978). However, these workers failed to observe any increased germination of conidia or increased germ-tube branching in the presence of either compound. They believed that the requirement for this compound was confined to an early stage of hyphal extension.

A simplified model of FEB resistance was proposed by Schroeder and Christensen (1963), who suggested that resistance to FEB was of two types; resistance to initial infection (Type I) and resistance to spread of the infection within the ear (Type II) (See Chapter 4). To assess cultivars for Type I resistance, ears were inoculated by spraying with a conidial suspension of *F. graminearum* and the number of infected spikelets were assessed as a measure of initial infection. Saur (1984) also found that four wheat cultivars differed in their resistance to initial infection following spray inoculation of

the entire ear with a conidial suspension of *F. culmorum*.

Little work at present has considered the biochemistry or morphological characters of anthers from different cultivars with respect to resistance to initial infection. Liang *et al.* (1981) related flower structure of individual wheat cultivars with resistance to FEB and observed that more susceptible cultivars retained their anthers inside the glume, whilst those exposing their anthers outside the glumes showed less symptoms of FEB. Takegami (1957a) found that dehisced anthers were essential for initial infection of wheat cultivars by *F. graminearum*. This author showed that cultivars differed in floral morphology with some cultivars having short anther filaments which were caught between the tips of closing glumes (Group A), whilst other cultivars held their anthers inside the glumes (Group B) (Takegami, 1957b). Observations suggested that those cultivars in Group A were more susceptible to infection during the early stages of flowering, whilst Group B were more susceptible during the early stages of grain filling. This may suggest why Schroeder and Christensen (1963) found that different cultivars of wheat were at their most susceptible to FEB at different stages of maturity.

The aim of this investigation was to observe whether anthers taken from a number of winter wheat cultivars of varying resistance to FEB showed any differential activity on the early growth of *F. culmorum in-vitro* that could account for differences in the Type I resistance proposed by Schroeder and Christensen (1963).

3.2 Materials and methods

3.2.1 Fungal material

An isolate of *F. culmorum* (416/10) (Table 2.2) of known pathogenicity which was obtained from wheat ears at Harper Adams was used in this study. Cultures were maintained and inoculum of 10^4 conidia ml^{-1} of water was prepared as in Chapter 2 (pages 34-36). This spore concentration meant germinating conidia were not too congested to allow detailed examination of branching germ-tubes under the microscope.

3.2.2 Preparation of anthers

Wheat anthers were obtained from field plots of five cultivar at anthesis GS 65 by shaking flowering ears into a labelled polythene bag and storing them in the freezer at -20°C until required. Anthers were specifically chosen according to weight. One hundred individual anthers were weighed for each wheat cultivar, using a microbalance and the mean calculated. For each cultivar, individual anthers were then re-weighed and those found within ± 0.01mg of the mean weight were chosen for our studies.

3.2.3 Effect of anthers on germination and preliminary growth of *F. culmorum* conidia

Anthers from five cultivars showing varying resistance to FEB (Avalon, Hussar, Riband, Mercia and Beaver) (Anon, 1995) (Table 2.1) were used in these experiments. During each experiment, ten single anthers of known weight were taken for each cultivar and placed into separate replicate 1 ml Eppendorff tubes. Ten μl of conidial suspension was then added to each anther before being incubated in darkness at 20°C. Dispensing 10 μl of conidial suspension into tubes with no anthers provided control treatments. Samples were fixed using 10 μl of lactophenol in cotton blue after 5 hours

to assess conidial germination and after 12 hours to assess germ-tube development.

All samples were prepared for examination under a light microscope by placing the anther and the entire 20μl contents of each Eppendorff tube onto a glass microscope slide and covering it with a cover slip. Initially, the number of pollen grains within each anther and in the surrounding solution were counted. The incidence of conidial germination was assessed (expressed as a percentage) on 50 conidia selected at random from each replicate tube. Conidia were considered to have germinated when germ-tubes were as long as the conidial spore was wide (Manners, 1966). The degree of branching of conidial germ-tubes was assessed by counting the number of terminal points on 25 germinated conidia selected at random for each replicate. Data was presented as the percentage of conidia with one, two, three, four, or more terminal points. Total mycelial length was assessed on five germinated conidia selected at random for each replicate by projecting the image from the eyepiece onto some drawing paper adjacent to the microscope using a camera lucida. The projected image of a germinated conidia could then be traced using a calibrated map reader so that the total length could then be calculated.

All data on the number of pollen grains per anther, % conidial germination, number of terminal points and total length of germ-tubes was analysed using analysis of variance. Regression analysis between the number of pollen grains for individual anthers with conidial germination, germ-tubes length and branching of *F. culmorum* conidia was also performed. All analysis was undertaken using the statistical package Genstat 5 version 3.2 (Lawes Agricultural Trust, Rothamsted, UK).

3.3 Results

3.3.1 Effect of anthers on germination of *F. culmorum.*

Analysis of variance revealed a significant difference ($P<0.01$) in the number of pollen grains per anther between wheat cultivars. Hussar showed the highest concentration of pollen grains with a mean of 52.3 grains per anther, whilst Riband had the lowest concentration of pollen grain with a mean of only 15.7 per anther (Table 3.1).

The percentage of germinated conidia was shown to increase significantly in the presence of anthers (Plate 3.1a) with up to 72.2 % of conidia having germinated in the presence of Hussar anthers compared to 9.2 % in controls (Plate 3.1b). No differences in the % germination of conidia were observed between cultivars. Regression analysis revealed no relationship between the number of pollen grains per anther and % germination of *F. culmorum* conidia.

Table 3.1. Percentage germination of *F. culmorum* conidia after 5 hours incubation at 20°C in the presence of individual anthers of five winter wheat cultivars.

Cultivar	Mean number of pollen grains per anther	%germination
Avalon	20.0bc	67.6a
Beaver	45.3ab	71.2a
Hussar	52.3a	72.2a
Mercia	24.9ab	72.0a
Riband	15.7c	70.2a
Control*	--	9.2b
s.e.d. (P<0.05),d.f. 45		
Cultivar x Control	--	2.3
Cultivar	10.1 (df 36)	3.0

* % germination of *F.culmorum* conidia in sterile distilled water only
For each cultivar numbers in each column followed by different letters are significantly different (P=0.05), according to the Duncans multiple range test.

3.3.2 Effect of anthers on preliminary growth of *F. culmorum.*

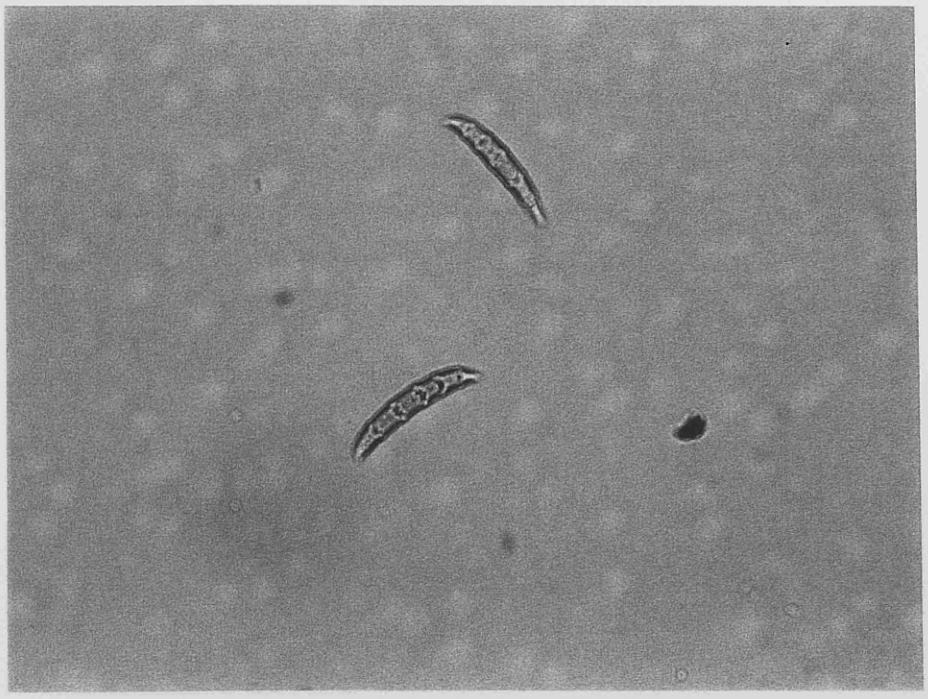
Observations on germinated conidia of *F. culmorum* in sterile distilled water revealed that the majority (60.4 %) produced only a single, thin, unbranched germ-tube (Plate 3.2a). In only a few cases (3.2 %) were germ-tubes branched producing up to three terminal points. However, in the presence of wheat anthers, conidia of *F. culmorum* produced thick germ-tubes which subsequently branched producing up to six terminal points (Plate 3.2b and c). Statistical analysis revealed significant (P<0.001) increases in the degree of branching of conidia and in total length of germ-tubes in the presence of anthers compared with the control.

The percentage of *F. culmorum* conidia showing one, two, three, four, or more terminal

points in the presence of anthers of each cultivar can be seen in Table 3.2. Analysis of variance revealed significant differences ($P<0.05$) between cultivars in the number of terminal points. For example, in the presence of anthers taken from Mercia, 34.4 % and 14.4 % of conidial germ-tubes possessed three or four terminal points, respectively. Conversely, in the presence of anthers from Hussar, the proportion of germ-tubes possessing three or four terminal points was only 25.2 % and 4.4 %, respectively.

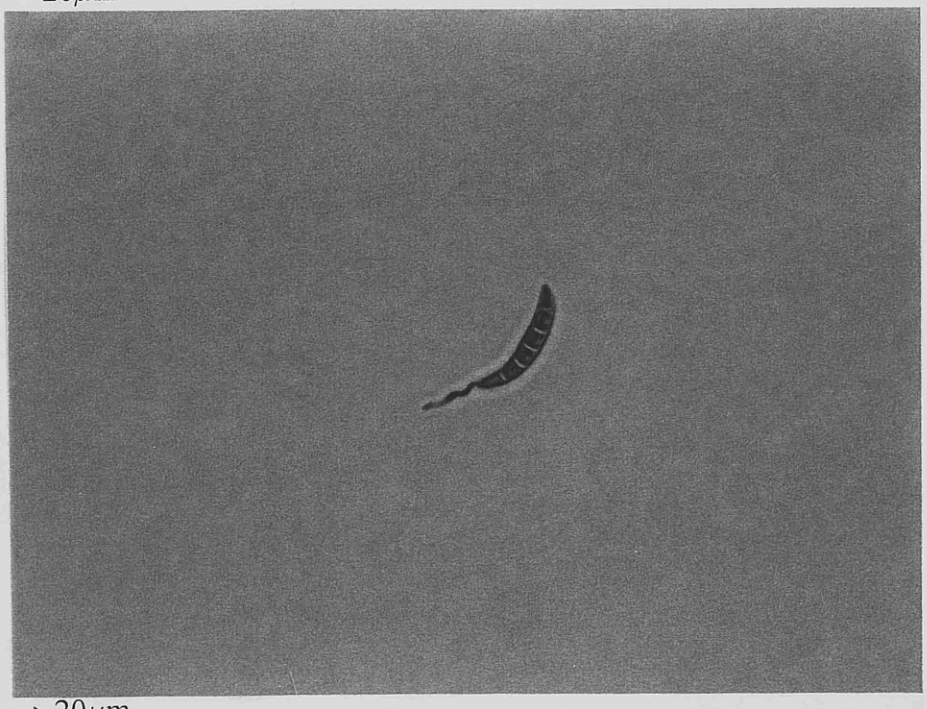
No significant differences were observed in total germ-tube length in the presence of anthers from different cultivars. Neither was any significant relationship observed between the total germ-tube length and branching of germ-tubes in these cultivars noted following regression analysis. Similar analysis also failed to reveal any significant relationship between the number of pollen grains per anther and total length of germ-tube.

(a)



◂---------▸ 20μm

(b)



◂---------▸ 20μm

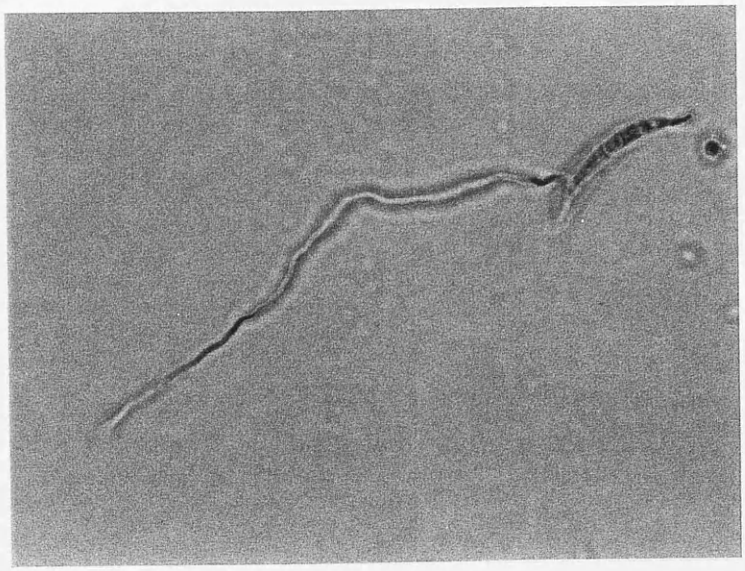
Plate 3.1 Light micrographs of germinating *F. culmorum* conidia 5 hours after incubation at 20°C in the presence of (a) sterile distilled water and (b) anthers of cultivar Avalon

Table 3.2 Percentage of *F. culmorum* conidia with one, two, three, four or more terminal points after 12 hours incubation at 20°C in the presence of anthers from five winter wheat cultivars.

Cultivar	Mean number of pollen grains per anther	Number of terminal points per conidium					Total germ-tube length (mm) per conidium
		1	2	3	4	>4	
Avalon	33.8c	8.8bc	55.6ab	26.4abc	8.8abc	0.8a	0.31ab
Beaver	49.9ab	6.0ab	44.0bc	32.8ab	12.8ab	2.0a	0.30b
Hussar	56.3a	10.0c	58.8a	25.2bc	4.4cd	0.4a	0.35a
Mercia	39.4bc	7.2a	41.2c	34.4a	14.4a	1.6a	0.28b
Riband	18.2c	11.2c	57.6a	23.2c	6.4bcd	0.8a	0.32ab
Control*	--	60.4a	36.4c	3.2d	0d	0a	0.09c
s.e.d. ($P<0.05$, d.f. 45							
Cultivar x Control	--	5.6	10.3	7.3	2.9	1.8	0.02
Cultivar	10.4 (df36)	7.2	13.3	9.4	7.4	2.4	0.02

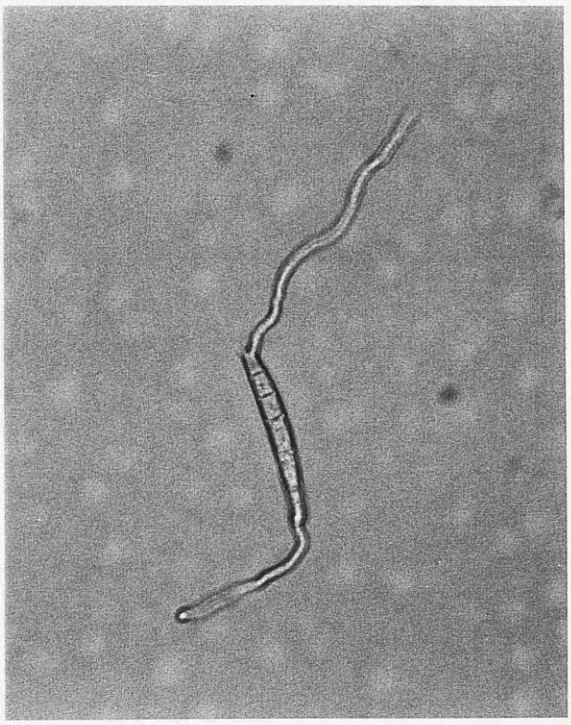
* % germination of *F.culmorum* conidia in sterile distilled water only
For each cultivar numbers in each column followed by different letters are significantly different (P=0.05), according to the Duncans multiple range test.

(a)



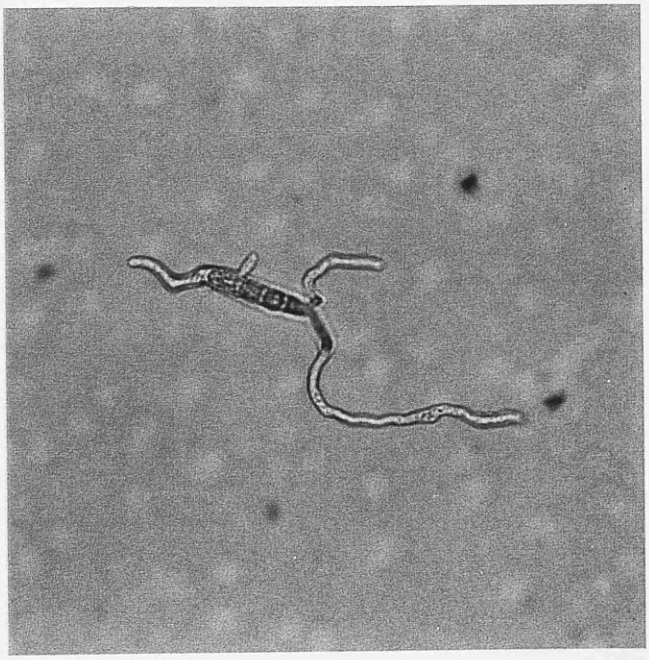
◂---------▸ 20μm

(b)



◂---------▸ 20μm

(c)



◂---------▸ 20μm

Plate 3.2 Light micrographs of germ-tube branching of *F. culmorum* conidia 12 hours after incubation at 20°C in the presence of (a) sterile distilled water, (b) anthers of cultivar Mercia, (c) anthers of cultivar Hussar.

3.4 Discussion

By placing individual anthers from the cultivars Avalon, Beaver, Hussar, Mercia and Riband into an Eppendorff tube with 10 μl of conidial suspension, differences in initial growth of *F. culmorum* were successfully studied. Nkongolo *et al.* (1993) used media amended with anther extracts of different genotypes of wheat to compare mycelial growth of *F. graminearum*. They found that extracts from the resistant wheat line Nobeoka Bozu stimulated growth significantly, while no growth occurred in the presence of extracts from the susceptible cultivar Laval-19. However, as mycelial growth is unlikely to reflect the early stages of initial infection, this method is not considered a useful technique to measure Type I resistance. Jenkinson (1994) used pollen suspensions of four different cultivars of winter wheat to compare germination and germ-tube length of conidia of a range of *Fusarium* spp and *M. nivale*. He revealed that pollen suspensions from the susceptible cultivar Avalon stimulated germination of *F. culmorum* and *F. avenaceum* significantly more than the less susceptible cultivar Longbow. This is in contrast with the present study where no significant difference in incidence of % germination was observed between cultivars. Unfortunately, Jenkinson (1994) did not quantify the anthers prior to making the pollen suspension so that anthers at different stages of maturity are likely to have been used. It is also likely that extracts may not have a direct bearing on the events that are taking place in the ears of wheat. In the present study, to ensure individual anthers were of similar maturity they were weighed prior to use and only those anthers within ± 0.01mg of the mean for that cultivar were used and only initial growth characteristics of *F. culmorum* conidia was measured.

The results from these experiments showed that anthers from the cultivars Mercia and Beaver caused significantly more germ-tube branching of conidia of *F. culmorum* compared with Riband and Hussar. As an increase in the number of germ-tubes can be regarded as an increase in the number of potential infection sites, these results suggest that Mercia and Beaver are more susceptible to initial infection than Hussar and Riband.

However, resistance of winter wheat cultivars to FEB in the field cannot be solely explained by differences in initial growth of *F. culmorum* conidia. According to the National Institute of Agricultural Botany, Avalon had a resistance score of 4 (Anon, 1989) on a scale of 1 (highly susceptible) to 9 (highly resistant) , whilst Hussar and Mercia had a resistance score of 5, with Riband and Beaver scoring 6 (Anon, 1995) (Table 2.1). Field trials at Harper Adams showed that the susceptible cultivar Avalon had the highest % of necrotic spikelets 5 weeks after inoculation with a mixture of *Fusarium* spp and *M. nivale*, whilst Mercia showed least symptoms, of the cultivars examined in this study (see Chapter 5). In terms of germ-tube branching Mercia had the highest level of branching, whilst Avalon and Hussar had the least. However, relating germ-tube branching to disease symptoms is complicated by the interaction between Type I and Type II resistance (Schroeder and Christensen, 1963).

Some workers have measured Type I resistance on different cultivars by spraying conidial suspension onto the ears of winter wheat and then assessing the number of infected spikelets (Schroeder and Christensen, 1963; Saur, 1984). Other workers have tried measuring Type I resistance by injecting inoculum into a number of individual

spikelets on each head and then recording the percentage of infected spikelets (Yong-Fang *et al.*, 1997). However, both of these methods rely on recording infection or visual symptoms which will be dictated not only by resistance to initial infection (Type I) but also resistance to colonisation (Type II) within the spikelet itself. It is, therefore, speculated that measuring the number of terminal points on germinating conidia of *Fusarium* spp or *M. nivale* in the presence of anthers from different cultivars of wheat may be a more reliable method of assessing Type I resistance. However, further work involving a greater number of cultivars, including resistant lines such as Sumai-3 and Nobeoka bozu, should be performed with a range of isolates of *Fusarium* spp and *M. nivale* before this method is recomended.

The basis of this increased germ-tube branching in the presence of anthers from certain cultivars is unclear. This work shows that stimulation of initial fungal growth could not be explained by the number of pollen grains per anther. It is, therefore, speculated that the factor or factors which stimulates fungal growth are associated with the anther rather than the pollen grains within. Although, Strange and Smith, (1978) showed that choline chloride and betaine hydrochloride significantly stimulated hyphal extension of *F. avenaceum, F. culmorum* and *F. graminearum,* it is unlikely that these compounds are the only stimulatory factors. For example, Ikeda *et al.* (1955) found increased concentrations of glucose and fructose in glumes of wheat and barley following anthesis. Although, Nkongolo *et al.* (1993) showed that mycelia of *F. graminearum* grew more as concentrations of betaine and choline were increased in amended media from 1 to 10^4 μM, when betaine was present at 0.1M it actually inhibited fungal growth. These workers also observed that neither of these compounds stimulated spore

germination of *F. graminearum*. However, the effect of these compounds on initial growth of *Fusarium* spp and *M. nivale* is at present unclear and further work to reveal the chemical basis of Type I resistance is required.

CHAPTER 4

Factors affecting resistance to colonisation by *Fusarium culmorum* and *F. graminearum* in ears of winter wheat.

4.1 Introduction

A number of assessment methods have been used to screen genotypes of winter wheat for resistance to FEB in the field. For example, Parry *et al.* (1984) devised a picture key which was used to assess the average percentage of the ears showing symptoms in the whole plot. A similar assessment system was used by Snijders (1990a), whilst Miedaner and Perkowski (1996) and Mesterhazy (1995) converted percentage scores to a numeric scale. In the UK, the National Institute of Agricultural Botany in their National and Recommended list trials produce a 1-9 scale where 9 is resistant and 1 is very susceptible to FEB (Anon., 1995).

Although these methods have revealed a large variability in resistance to FEB among wheat genotypes, little work has studied the mechanisms controlling resistance. Schroeder and Christensen (1963) believed that at least two major factors were involved in the resistance of wheat cultivars to FEB: resistance to initial infection (Type I resistance) and resistance to the spread of infection within the ear (Type II resistance). After injecting a conidial suspension of *F. graminearum* into the medial spikelets of seven cultivars of winter wheat, Schroeder and Christensen (1963) measured the number of infected spikelets within individual ears in order to identify variation of Type II resistance. They found that although cultivars could exhibit Type II resistance, they may not necessarily also exhibit Type I resistance and *vice versa*. Point inoculating individual spikelets with *F. graminearum* has also been used to identify lines of *Triticum* resistant to spread of infection amongst 1076 accessions in China (Yong-Fang *et al.*, 1997).

Symptoms of FEB are first observed as small brown water soaked lesions at the base of the outer glumes. Complete necrosis of individual spikelets then occurs before symptoms spread to the rachis, then to adjoining spikelets before the advancing infection usually proceeds down the ear (Bai and Shaner,1996b). The spread of infection by *F. culmorum* in different cultivars of wheat has been closely related to DON content in the chaff and kernels (Snijders and Kretching,1992). In some cultivars, rapid bleaching (scalding) above the point of infection is also a common feature but it is not known if this symptom is due to infection and, therefore, an indication of Type II resistance or whether it is a secondary symptom related to infection. Atanasoff (1920) believed that this scalding of the healthy part of the ear was due to the blocking of the vascular bundles in the rachis by the fungus which cut off the water and food supply. Field observations by the author have indicated that some cultivars are more inclined to show scalding than others.

This study aimed to identify explanations for the basis of type II resistance by inoculating single spikelets with *F. culmorum* and *F. graminearum* under glasshouse conditions. By relating symptoms of necrosis and scalding to fungal colonisation it was hoped to establish how these symptoms are related to Type II resistance.

4.2 Materials and methods

4.2.1 Inoculation and disease assessment

Cultures of *F. culmorum* (416/10) and *F. graminearum* (F94-8) were maintained and inoculum of 2.5 X 10^5 conidia ml^{-1} of water was prepared as in Chapter 2 (pages 34-36). Plants were maintained as in Chapter 2 (pages 32-34) and soil was kept moist at all times by watering from the base of the pots. Each cultivar was inoculated individually at mid-anthesis when 75 % of ears were at GS 65 by placing 25μl of spore suspension, using a pipette, between the lemma and palea of spikelet ten (counting from the base of the ear) of individual ears. Following inoculation, clear plastic bags were placed over each ear for 48 hours in order to maintain high humidity for the infection and development of disease. Four weeks after inoculation the number of spikelets showing necrosis and scalding in the distal and proximal parts of the ear (Figure 4.1) was recorded. Scalded spikelets were identified as pale and almost white in colour but which did not exhibit any symptoms of necrosis.

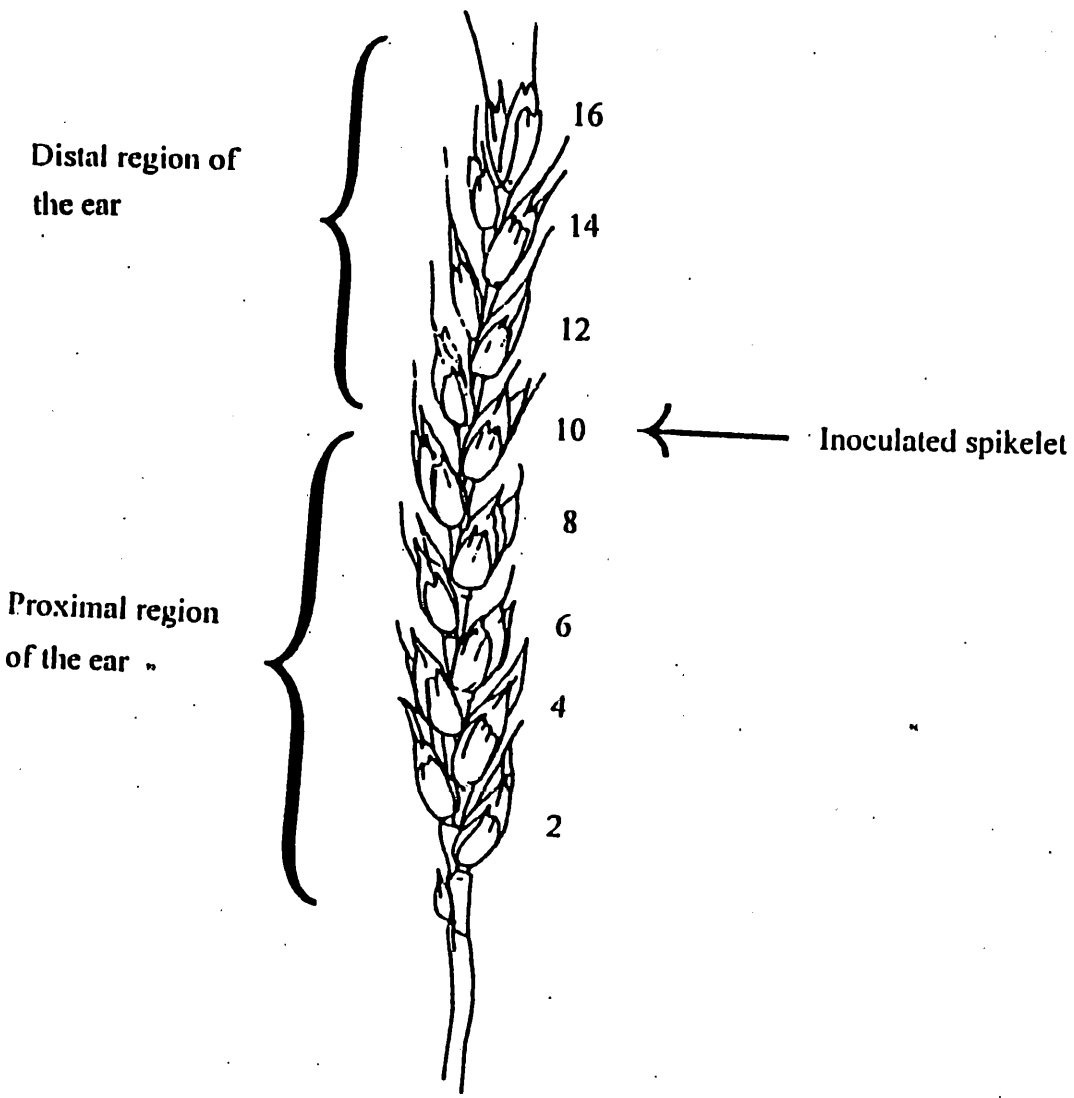


Figure 4.1 Diagrammatic representation of the inoculated spikelet (10th spikelet from the base of the ear) showing the distal (above the point of inoculation) and proximal (below the point of inoculation) regions of the ear.

4.2.2 Glasshouse experiment 1- Symptom development in different cultivars of winter wheat

Twelve, NIAB recommended cultivars (Admiral, Apollo, Avalon, Beaver, Brigadier, Genesis, Haven, Hereward, Hunter, Hussar, Mercia and Riband) of winter wheat (Anon, 1995) (Table 2.1) were grown as in Chapter 2 (pages 32-34) and laid out in a random block design with ten replicate pots each. At mid anthesis, six ears from each pot were tagged; two were inoculated with a conidial suspension of *F. culmorum*, two were inoculated with *F. graminearum* and two were treated with water as controls.

In order to analyse data, the number of spikelets showing necrosis and scalding in both the distal and proximal parts of the ear was converted into a percentage by dividing with the total number of spikelets in each of these regions of the ear and multiplying by 100. Two way analysis of variance was then performed to compare differences in % necrosis and scalding between individual *Fusarium* spp, followed by one way analysis of variance for individual *Fusarium* spp to compare differences between cultivars. Regression analysis was then used to assess the strength of the relationship between necrosis in the proximal region and scalding in the distal region of the ear. As the % of spikelets showing scalding in the distal half of the ear was binomially distributed, this data was fitted to a binomial logistic model, with the total number of spikelets in the distal region as the binomial totals and the number of scalded spikelets as the response variate. Spearman rank correlations were also performed between necrosis in the proximal region and scalding in the distal region of the ear. All analysis was done using Genstat 5, Release 3.2 (Lawes Agricultural Trust, Rothamsted, UK).

4.2.3 Glasshouse experiment 2 - relating symptom development to fungal colonisation

Five cultivars (Sumai-3, Ringo Star, Riband, Virtue and Kraka) of winter wheat (Table 2.1) were grown as in Chapter 2 (pages 32-34) and laid out in a random block design with five replicate pots. Four ears from each pot were then inoculated with *F. culmorum* before being assessed for the number of spikelets showing necrosis and scalding 4 and 6 weeks later. To understand if the symptoms of necrosis and scalding could be related to fungal colonisation by *F. culmorum,* the incidence of infection was assessed by re-isolating the fungus from spikelets and rachis segments. Two replicate ears from each pot were selected at random, 4 and 6 weeks after inoculation. Ears were then detached and dissected into individual spikelets and rachis segments which were surface sterilised by washing in 5 % sodium hypochlorite solution (0.5 % available chlorine) for 20 seconds before being rinsed in two changes of sterile distilled water. All rachis segments and spikelets were then placed onto PDA and incubated in darkness for 48 hours at 20°C. Infection was then assessed by observing colonies of *F. culmorum* emerging from the plant segments. The relationship between the number of necrotic spikelets and the number of spikelets infected with *F. culmorum* was investigated by regression analysis on individual cultivars.

4.3 Results

4.3.1 Experiment 1 - Symptom development in different cultivars of winter wheat

Initial water-soaked lesions appeared on the outer glumes 7 days after inoculation; within 10 days the spikelets appeared completely necrotic and by 14 days, necrosis was seen to progress to adjacent spikelets above and below the point of inoculation via the

rachis. In all cultivars, necrosis developed predominantly in the proximal (below spikelet 10) end of the ear (Figure 4.2) (Plate 4.1b) with ears having an average of 25 % of spikelets showing necrosis in the proximal half of the ear but only 3 % in the distal section (Table 4.1 and 4.2). Three weeks after inoculation, premature bleaching was also observed in the distal (above spikelet 10) end of the ear directly above the necrotic spikelets below (Plate 4.1c), but not in the proximal section of any ear. No symptoms of necrosis or scalding were observed in the controls.

Inoculation with *F. culmorum* resulted in a significantly ($P<0.001$) higher percentage of spikelets showing necrosis and scalding in the proximal and distal region of the ear than those inoculated with *F. graminearum* (Table 4.1 and 4.2). For example, an average of 30 % of spikelets found within the proximal part of the ear showed necrosis following inoculation with *F. culmorum*, whilst those inoculated with *F. graminearum* showed an average of 20 %. Similarly, an average of 16 % of spikelets in the distal part of the ear showed scalding following inoculation with *F. culmorum*, whilst those ears inoculated with *F. graminearum* showed an average of 7 % scalding.

For ears inoculated with *F. culmorum,* the percentage of spikelets showing necrosis in the proximal region of the ear differed significantly ($P<0.01$) between wheat cultivars (Table 4.1). In the cultivar Admiral, a mean of 7.5 % of spikelets in the proximal half of the ear showed necrosis, with symptoms often being restricted to the inoculated spikelet (Plate 4.1a). In contrast, the cultivar Riband had the highest proportion of necrotic spikelets below the point of inoculation with a mean of 49.5 % and had necrosis spreading down the ear and in some cases even affecting the top of the peduncle (Plate

4.1b).

Cultivars also differed significantly ($P<0.05$) in the percentage of spikelets showing scalding following inoculation with *F. culmorum* and *F. graminearum*. Following inoculation with *F. culmorum*, Hussar had the greatest number of scalded spikelets (39.6 %), whilst Mercia and Admiral showed no scalding. Hussar also had the most scalding (24.8 %) following inoculation with *F. graminearum*, whilst Admiral, Beaver, Genesis and Mercia showed no scalding.

Regression analysis revealed a positive relationship between the percentage of spikelets showing necrosis in the proximal part of the ear and the percentage of scalded spikelets in the distal part of the ear following inoculation with either *F. culmorum* ($P<0.001$) (Appendix 1) or *F. graminearum* ($P<0.001$) (Appendix 2). Spearman rank correlation also revealled a significant ($P<0.05$) association between the percentage of spikelets showing necrosis in the proximal part of the ear and the percentage of scalded spikelets in the distal part of the ear following inoculation with *F. culmorum* but not with *F. graminearum*. Following inoculation with *F. graminearum*, cultivars differed significantly in the amount of scalding ($P<0.001$), whilst a significant interaction ($P<0.05$) between the percentage of spikelets showing necrosis indicated that the severity of necrosis required before scalding occurred differed between cultivars. For example, although Brigadier and Hereward showed a similar number of necrotic spikelets, Brigadier had a significantly higher proportion of scalded spikelets. However, no significant cultivar effect or an interaction between the percentage of spikelets showing necrosis and cultivar was observed following inoculation with *F. culmorum*.

Table 4.1 Percentage of the proximal and distal regions of ears in 12 cultivars of winter wheat showing necrosis and scalding 4 weeks after inoculation with *F. culmorum*. Spikelet ten (counting from the base of the ear) was point inoculated with 25μl of conidial suspension at GS 65.

Cultivar	NIAB score[1]	% necrosis		% scalding	
		distal	proximal	distal	proximal
Admiral	5	2.6	7.5	0	0
Apollo	6[2]	2.5	15.5	12.9	0
Avalon	4[2]	2.4	22.0	13.7	0
Beaver	6	4.6	37.0	6.5	0
Brigadier	6	1.7	46.5	23.3	0
Genesis	6	6.0	24.0	19.1	0
Haven	5	3.0	29.0	13.1	0
Hereward	6	5.5	35.5	27.6	0
Hunter	5	6.3	29.5	17.3	0
Hussar	5	3.1	41.5	38.6	0
Mercia	5	1.7	28.0	0	0
Riband	6	5.6	49.5	18.4	0
s.e.d. ($P<0.05$), d.f. 99		NS	10.8	9.6	NS

[1] - Assessed on a scale of 1 (highly susceptible) to 9 (highly resistant)
[2] - (After Anon., 1989

Table 4.2 Percentage of the proximal and distal regions of ears in 12 cultivars of winter wheat showing necrosis and scalding 4 weeks after inoculation with *F. graminearum*. Spikelet ten (counting from the base of the ear) was point inoculated with 25μl of conidial suspension at GS 65.

Cultivar	NIAB score[1]	% necrosis		% scalding	
		distal	proximal	distal	proximal
Admiral	5	1.0	20.0	0	0
Apollo	6[2]	5.2	14.0	4.5	0
Avalon	4[2]	0.4	12.0	4.2	0
Beaver	6	2.5	24.0	0	0
Brigadier	6	1.8	26.5	17.3	0
Genesis	6	6.2	10.4	0.3	0
Haven	5	3.6	20.0	5.0	0
Hereward	6	1.5	25.5	9.8	0
Hunter	5	1.1	19.5	10.0	0
Hussar	5	3.6	34.0	24.8	0
Mercia	5	0.4	16.0	0	0
Riband	6	2.3	13.5	4.6	0
s.e.d. ($P<0.05$), d.f. 99		NS	NS	7.0	NS

[1] - Assessed on a scale of 1 (highly susceptible) to 9 (highly resistant)
[2] - (After Anon., 1989

(a)

(c)

(b)

Plate 4.1 Symptoms of FEB 4 weeks after point inoculation of spikelet 10 (counting from the base of the ear) at mid-anthesis with a conidial suspension of *F. culmorum*. (a)Shows in cultivars like Apollo, necrosis was often confined to the inoculated spikelet, whilst (b) in more susceptible cultivars, like Riband, necrosis spread down the ear affecting an average of 6 spikelets. (c)Shows in severe cases, such as in this ear of Brigadier, necrosis (brown in colour) caused scalding (paler in colour) above the point of inoculation. Arrows indicate inoculated spikelet.

4.3.2 Experiment 2 - relating symptom development to fungal colonisation

There were significant differences ($P<0.01$) in number of necrotic spikelets, as well as spikelet ($P<0.001$) and rachis infection ($P<0.01$) between cultivars. In the most resistant cultivar, Sumai 3, necrosis was restricted to the inoculated spikelet, whilst Virtue was the most susceptible and had an average of seven spikelets showing necrosis (Table 4.3). Regression analysis showed that in resistant cultivars, such as Sumai 3 and Kraka, the number of necrotic spikelets corresponded directly to the number of infected spikelets ($P<0.001$). In more susceptible cultivars, such as Virtue, there was also a significant, ($P<0.001$) but weaker relationship ($R^2 = 0.52$) due to symptomless infection in the distal half of some ears (Appendix 3). Significant relationships ($P<0.001$) between rachis infection with number of necrotic and infected spikelets were also observed. For example, Riband and Kraka showed strong relationships between spikelet necrosis and rachis infection ($R^2 = 0.93$ - 0.99 and 0.88 respectively), whilst weaker relationships were shown by Ringo - Star and Virtue ($R^2 = 0.58$ and 0.63 respectively) as rachis infection was more advanced than spikelet necrosis.

Table 4.3 Mean number of necrotic spikelets, infected spikelet and rachis segments, 6 weeks after artificial inoculation of the 10th spikelet from the base with *F. culmorum*. Number of infected spikelets and rachis segments was assessed by re-isolating the fungus onto PDA.

Cultivar	Number of necrotic spikelets	Number of infected spikelets	Number of infected rachis segments
Sumai 3	1.0	0.8	0.9
Ringo star	5.0	3.8	7.4
Riband	4.3	4.8	5.8
Virtue	7.0	9.8	6.1
Kraka	2.1	2.3	3.0
s.e.d. ($P<0.05$), d.f. 28	4.0	4.6	4.8

4.4 Discussion

Inoculation of spikelet 10 in the middle of the ear, enabled a detailed study of infection by *F. culmorum* and *F. graminearum* on symptom development of individual cultivars of winter wheat. The study has shown that although symptoms of necrosis and scalding may visually appear similar (Plate 4.1) they are in fact both distinct, with necrosis being related to infection by the pathogen (Table 4.3), whilst scalding is a secondary symptom associated with severe necrosis (Table 4.1 and 4.2). This would suggest that workers in the past have overestimated disease severity of disease, especially in more susceptible cultivars which are likely to show scalding following inoculation with *Fusarium* spp and *M. nivale*. Indeed, cultivars differed significantly in severity of scalding following inoculation with both *F. culmorum* and *F. graminearum*. Although scalding occurred in most cultivars, some required a greater number of necrotic spikelets before scalding could occur following inoculation with *F. graminearum*. Similar findings were not

found following inoculation with *F. culmorum*.

The successful re-isolation of *F. culmorum* from inoculated ears revealed that necrotic symptoms were a direct result of the inoculation procedure. However, since *F. culmorum* was isolated from only a limited number of scalded spikelets it would appear that measuring spikelet necrosis is a more appropriate method of measuring Type II resistance (Schroeder and Christensen, 1963). This implies that cultivars such as Admiral and Apollo, which showed only a limited number of necrotic spikelets following inoculation with *F. culmorum,* were more resistant to colonisation within the ear (Type II) than Brigadier and Riband, which showed a high percentage of spikelets showing necrosis. This study, therefore, confirms that this technique is a useful method to measure Type II resistance as previously used by Saur (1984) and Yong Fang *et al.* (1997). However, further experiments are required to test the robustness of this method under a range of different environmental conditions, including different temperatures and watering regimes.

Significant differences between cultivars in necrosis and scalding were observed following inoculation with *F. culmorum*, but only differences in scalding were observed following inoculation with *F. graminearum*. This demonstrates the more aggressive nature of *F. culmorum* compared with *F. graminearum* in terms of infection in the ear (Table 4.1 and 4.2). Arsenuik *et al.* (1993) found that disease severity was higher in field plots of 14 cultivars of winter cereals (triticale, wheat and rye) following inoculation with a conidial suspension of *F. culmorum* than *F. graminearum*. Similarly, Wong *et al.* (1995) showed that there was a higher proportion of shrivelled (tombstone)

grains among 11 winter wheat cultivars, grown in a field experiment, that had been artificially inoculated with a spore suspension of *F. culmorum* than *F. graminearum*. Turner and Jennings (1997), whilst looking at the effect of different *Fusarium* species and *M. nivale* on yield loss of winter wheat also noted differences in symptom development. Among *F. culmorum* and *F. graminearum* a single site of infection could cause total loss of grain production above the point of infection, probably due to scalded spikelets. However, similar infection by *F. avenaceum, F. poae* or *M. nivale* remained confined to the infected spikelet, having little effect on grain development.

The implications of scalding in terms of yield loss and reduction in grain quality are at present unclear. Atanasoff (1920) commented that where scalding occurred in the top half of the ear, spikelets shrink and fail to develop normally compared with uninfected ears. This would imply that although scalded spikelets are not directly affected by infection, they may cause significant yield losses due to the formation of shrivelled grain. Although DON content was shown to be closely related to infection by *F. culmorum* (Snijders and Krechting, 1992) it is not known whether scalded spikelets are similarly related to mycotoxin production.

Severity of necrosis and scalding within inoculated ears of individual cultivars in these experiments could not be related to susceptibility observed in the field. According to the National Institute of Agricultural Botany, Avalon had a resistance score of 4 (Anon, 1989) on a scale of 1 (highly susceptible) to 9 (highly resistant) , whilst Mercia had a 5, with Riband and Brigadier scoring 6 (Anon, 1995). In this experiment, many cultivars that are susceptible under field conditions, such as Avalon (Parry *et al.*, 1984) were

relatively resistant to colonisation and scalding. The susceptibility of Avalon in the field may be explained by its susceptibility to initial infection (Type I) (Chapter 3) rather than the spread of the pathogen within the ear (Type II) (Schroeder & Christensen, 1963). It can be concluded, therefore, that symptoms in the field are the result of an interaction between Type I and Type II resistance (Schroeder and Christensen, 1963). Thus, although Riband had the most severe necrosis following inoculation with *F. culmorum* in this work, it scored a relative high score of 6 according to the National Institute of Agricultural Botany (Anon, 1995). This could partly be explained by anthers of Riband failing to encourage excess germ-tube branching in *F. culmorum* which is believed to be related to Type I resistance (Chapter 3). Similarly, Gilbert *et al.*(1997) found that there was no correlation between severity of FEB following inoculation with *F. graminearum* by spraying and injection of a single spikelet in twenty wheat plants derived from crosses between resistant and susceptible parents. It was concluded that these inoculation methods expressed different genetic responses. Spray inoculation infects a number of florets and hence numerous infection sites are possible if that cultivar is type I susceptible. However, inoculation of individual spikelets relies on a single site of infection and although initial symptoms depend on type I resistance, spread of necrosis is clearly caused by type II resistance. Thus Gilbert *et al.*(1997) found that using spray inoculation caused a wider range of symptoms and they proposed that this method should be used to measure cultivar resistance in the field. Recently, attempts have been made to breed wheat cultivars to both Type I and II resistance (Gilchrist *et al.*, 1997). These workers have observed that although cultivars such as Frontana possess both type I and II resistance, many genotypes possess only one type.

The physiological basis for Type II resistance is at present unclear. Early histological studies of rachis sections from a number of cultivars failed to reveal any association between anatomical characters and resistance to the spread of infection (Schroeder and Christensen, 1963). Other histological studies looking at vascular bundles in ears of winter wheat, show that spikelets in the distal region of the ear are supplied by smaller bundles suggesting that assimilate movement to the distal spikelets is reduced compared with the basal spikelets (Whingwiri *et al.*, 1981). This could explain why infection by *F .culmorum* spreads downwards towards the increased concentration of assimilate in the proximal half of the ear, leaving the distal region above the point of infection unaffected. Whether bundle size varies between cultivars and whether this could affect resistance to colonisation is not known. However, source sink-relationships within the ear are not well understood and other factors such as partitioning of assimilate between (Slafer and Savin, 1994) and hormonal control (Patrick, 1976) could also be important. A histological investigation on a range of cultivars of wheat, to study differences in the vascular tissue which could be related to resistance to spread of infection (Type II) is therefore suggested.

One compound that is believed to be related to the spread of infection is the tricothecene, DON. Miller *et al.* (1985) found that ears from resistant cultivars of spring wheat, triticale and rye contained low concentrations of DON and ergosterol, whereas susceptible cultivars of wheat and triticale contained higher concentrations of both of these following inoculation with *F. graminearum*. They believed that high concentrations of ergosterol in the susceptible cultivars showed that they had lower resistance to hyphal invasion. Unfortunately, as this analysis was not species specific,

no account was taken of contamination by other fungal species. However, other workers have also found reduced concentration of DON in resistant cultivars of wheat (Teich *et al.*, 1987; Snijders and Perkowski, 1990; Snijders and Kretching, 1992; Atanassov *et al.*, 1994; Wong *et al.*, 1995). The reduced level of DON in resistant cultivars is partly due to inhibited translocation of this compound in these genotypes. Snijders and Krechting (1992) showed that in a resistant line, translocation of DON from chaff to grain was greatly reduced compared with a susceptible host. As a result, it was concluded that a membrane-based tricothecene tolerance may be in operation in resistant genotypes. Despite there being a significant relationship between resistance to FEB and DON content in grain from various cultivars of wheat, a number of resistant cultivars have shown high DON content, whilst some susceptible cultivars have shown low DON content (Teich *et al.*, 1987). Such results have lead people to believe that there are cultivar specific effects that can influence the concentration of DON in the grain (Snijders and Perkowski, 1990) suggesting this is not the only factor determining levels Type II resistance.

The importance of these mycotoxins in terms of pathogenicity is unclear. However, recent work to determine if trichothecene production contributes to the virulence of *F. graminearum* have used tricothecene-deficient mutants which were developed by disrupting the *Tri5* gene that encodes the enzyme trichodiene synthase, which catalyses the first step in tricothecene biosynthesis (Proctor *et al.*, 1995). When a total of five cultivars of wheat, in two separate field trials, were artificially inoculated with a tricothecene non-producing isolate of *F. graminearum* they consistently showed reduced disease severity and incidence when compared to the wild-type isolate (Desjardins *et al.*,

1996). Using mutant isolates to compare symptoms of necrosis and scalding following inoculation of individual ears would lead to a greater understanding of the importance of DON in terms of symptom development and resistance to colonisation (Type II). It is therefore recommended that such an experiment should be performed in the future.

It is concluded that symptoms of FEB on different cultivars in the field are not only due to Type I and II resistance as proposed by Schroeder and Christensen (1963) but also scalding. Further work is required to identify the mechanisms responsible for the spread of necrosis and scalding.

CHAPTER 5

Relationship between cultivar morphology and severity of Fusarium ear blight in winter wheat

5.1 Introduction

Early work on the relationship between morphology of wheat cultivars and susceptibility to FEB failed to reveal any characters that could be correlated with disease severity (Christensen *et al.*, 1929). More recently, Couture (1982) found a significant negative correlation between cultivar height and seed infection by *Fusarium* spp. in 13 lines of wheat in Quebec, Canada. Recent work by Mesterhazy (1995) showed that in a naturally infected field trial, short strawed cultivars of wheat (below 70cm) tended to have more symptoms of FEB than taller cultivars (above 1m). However, when ears of the same cultivars were artificially inoculated under glasshouse conditions, no significant differences in disease severity were observed. It was speculated, therefore, that the differences in disease resistance between cultivars was due to disease escape, where taller cultivars have ears held at greater distances from the primary sources of inoculum such as infected crop debris. Whether short cultivars would have more symptoms than taller cultivars following artificial inoculation with *Fusarium* spp in the field was not tested.

Other morphological characters of winter wheat have also been related to severity of FEB. Awned cultivars were shown to have 80 % more symptoms of FEB in naturally infected field trials than non-awned cultivars (Mesterhazy, 1989). It was suggested that awned cultivars have a larger ear surface for airborne conidia to encounter and dew to form on (Mesterhazy, 1995). Compact, erect ears have also been found to have more severe symptoms of FEB than those with loose 'nodding' ears (Mesterhazy, 1995). There has been little published work on the relationship between other morphological characters such as peduncle length and leaf area and the severity of FEB

These experiments aimed to reveal morphological features that could be associated with resistance to FEB among wheat cultivars under artificially inoculated conditions. Further experiments were established to study the basis of the relationship behind cultivar height and severity of FEB by producing hybrids of contrasting height and resistance, studying the progeny and comparing the microclimate at ear height within a short and tall genotype.

5.2 Materials and methods

5.2.1 Production of inoculum

Conidial suspensions (concentration 2.5 X 10^5 ml^{-1} of water) of four isolates including one from each of the following species; *F. avenaceum* (F94-6), *F. culmorum* (F90-2), *F. graminearum* (F94-8), and *M. nivale* (F91-3) obtained from Plant Breeding International (Cambridge) (Table 2.2) were used in all field experiments and prepared as in Chapter 2 (pages 34-36).

5.2.2 Inoculation and disease assessment of field plots

Equal volumes of each conidial suspension were dispensed into a knapsack sprayer (Bastion 15, Application technique Ltd, Herts, UK). The spore mixture was then applied directly onto the ears of wheat plants at GS 65 at a rate of 35 ml^{-1} /m^2. Immediately after inoculation, overhead mist irrigation (Access irrigation, Northampton, UK) was applied to plots for a period of 3 minutes every 30 minutes (between 0800 and 1800 hours) to provide conditions conducive to infection. Mist irrigation was continued for 5 weeks after inoculation (Plate 5.1).

Plate 5.1 Overhead mist irrigation of field plots at Plant Breeing International, Cambridge, in June 1995, following inoculation at GS 65 with a mixture of *Fusarium* spp and *M. nivale*. Irrigation was applied at a frequency of 3 minutes every half hour between 8.00am and 8.00pm for 5 weeks after inoculation.

Disease severity was assessed 5 weeks after inoculation by counting the number of necrotic spikelets within an ear and expressing this as the % of total spikelets showing necrosis.

5.2.3 Field experiments

Four experiments compared disease and height following artificial inoculation, two at Cambridge and two at Harper Adams. A further experiment at Harper Adams in 1996/97 compared humidity in tall and short isogenic lines.

5.2.4 Experiment 1 - Analysis of cultivars

Seventeen cultivars of winter wheat were sown on 10/ 10/ 1995 at Harper Adams at a rate of 350 seeds/m^2 in plots 6 x 3m according to a fully randomised block design with three replicate blocks. A central quadrat (2m x 2m) of each plot was artificially inoculated when plants were at GS 65. Twenty tillers, selected at random, were removed from each plot and the mean tiller height, peduncle length (mm), leaf area index (LAI) and compactness of the ear were calculated. The LAI per plot was calculated by firstly measuring the total leaf area (cm^2) with a leaf area metre (Delta-T Devices Ltd, Cambridge, UK) and multiplying by the average number of tillers per m^2 which was measured in three replicate counts per plot Compactness of the ear was assessed as the number of spikelets per cm length of rachis. Five weeks post - inoculation, the % of spikelets showing necrosis for 50 ears from the inoculated quadrat was determined for each plot. Controls consisted of uninoculated sections of the field plots.

5.2.5 Experiment 2 - Analysis of segregating populations

The F_3 progeny of two crosses between parents of different straw height were studied at PBI, Cambridge. Cross 1 consisted of the tall strawed cultivar Kraka crossed with a short strawed line derived from an F_7 line (Konsul x Encore). Cross 2 consisted of Kraka crossed with the short strawed cultivar Charger. For each cross, 72 lines including parents were sown as F_2 established plants in 1994. $_3F$ grain from each plot was harvested in August 1995 and resown in November in separate plots (90cm x 90cm) arranged in a randomised block design with three replicates. Seed was hand sown to produce square plots of five by five plants. Height was measured as the distance from soil level to the top of the ear on the main shoot at F_2 and the main type in each F_3 plot. Date of anthesis was recorded as days after May 31 when 50 % of each individual plot was flowering. Disease severity was recorded as in Experiment 1.

5.2.6 Experiment 3 - Analysis of isogenic lines of Maris Huntsman and Maris Widgeon differing in the allelic form of the dwarfing gene *Rht1*

During October 1995, eight near-isogenic lines of both Maris Huntsman and Maris Widgeon differing in the allelic form of the dwarfing gene *Rht1* (Flintham *et al.*, 1997) were sown at Cambridge, in October, at a rate of 350 seeds/m^2 in a randomised block design with four replicates in plots 2m x 1m in size. Duplicates (a and b) for each allelic form were included. The following summer, plot height (cm) and severity of FEB following artificial inoculation were assessed.

5.2.7 Experiment 4 - Analysis of isogenic lines of Maris Huntsman differing in the allelic form of the dwarfing genes *Rht1* and *Rht2*

During November 1996 season, eight lines of Maris Huntsman differing in the allelic form of *Rht1* and *Rht2* (Flintham *et al.*, 1997) were sown at Harper Adams at a rate of 350 seeds/m^2 in a randomised block design with four replicate plots (3m x 3m). Duplicates (a and b) for each allelic form were included. A central area (1m x 1m) of each plot was used to assess the % of spikelets showing necrosis on 50 individual tillers, whilst 20 plants taken randomly from around the central area were used to determine mean tiller height.

5.2.8 Experiment 5 - Measurement of relative humidity in a short and tall line of Maris Huntsman

Two replicate plots 3m x 3m of both a short (containing *Rht1*) and tall (containing *rht1*) isogenic lines of Maris Huntsman were established at Harper Adams during 1996 /97. Within each plot, two calibrated humidity sensors (Skye 2010, Llandrindod Wells, UK) were placed placed at ear height 1m in from both ends. Polystyrene cups with holes cut in the side to maintain air movement were placed over the top of the sensors to protect them from excess rainfall and irrigation water. Data was collected every hour and stored using a data logger (Delta-T Devices Ltd, Cambridge, UK).

5.2.9 Analysis of data

Differences between genotypes in morphological characters and disease symptoms were subjected to analysis of variance, regression analysis and spearmans rank correlation. As residuals plots of disease severity in experiment 1 indicated that this data was not

normally distributed it was angularly transformed prior to analysis. Differences in humidity between the tall and short lines of Maris Huntsman was initially assessed. To look for differences in humidity over time average daily humidity was calculated and then area under the humidity curve was asessed. All analysis was carried out using the statistical package Genstat 5, version 3.12 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK).

5.3 Results

5.3.1 Experiment 1 - Analysis of cultivars

Although wheat cultivars differed significantly in straw height, peduncle length, LAI and compactness of ear according to analysis of variance ($P<0.001$) (Table 5.1), straw height, compactness of ear and LAI were the only morphological characters which were shown to have a significant association with severity of FEB according to linear regression analysis (Appendix 4). Significant ($P<0.05$) associations were also observed between straw height, LAI and compactness of ear with severity of FEB. The most significant relationship established was that between cultivar height and disease severity where over 30 % of the variance was accounted for. Overall, disease severity was more severe on shorter strawed cultivars than on taller strawed cultivars (Figure 5.1). For example, the short cultivars, Virtue and Haven had high disease severities, with 50 and 44 % of spikelets showing necrosis, respectively, whilst the tall cultivars, Cadenza, Kraka and Spark all had less than 10 % spikelets showing necrosis. However, if the outlying cultivar Kraka was removed from the regression analysis the percentage of variance accounted for was reduced to 13 % (equation $y = 103.6 - 0.91x$, $P< 0.01$).

The relationship between compactness of ear and disease severity showed that those cultivars with a more lax ear (low value of spikelets per cm rachis) had less symptoms of FEB than those with a denser ear (high value of spikelets per cm of rachis)(Figure 5.2). For example, the 'open eared' cultivar Spark had only 4 % spikelets showing necrosis compared with the 'compact eared' cultivar Avalon with 48 %. However, it was also observed that cultivars possessing ears with the same degree of compactness showed varying degrees of disease severity. For example, the cultivars Haven and Kraka, which both exhibited ears possessing an average 2.5 spikelets per cm rachis, showed 44 and 6 % spikelets infected by *Fusarium,* respectively. Such observations explained the weak relationship between compactness of ear and FEB determined by linear regression analysis where only 22 % of variance was accounted for (Appendix 4). When the outlying cultivar Spark was removed from the regression analysis the percentage of variance accounted for was reduced to 15 % (equation $y = 10.6 - 29.3x$, $P < 0.001$)

The relationship between LAI and severity of FEB indicated that those cultivars with denser leaf canopies tended to have more severe symptoms of FEB. However, this was a weak relationship and LAI could only account for 12 % of variation in severity of FEB (Appendix 4). A similarly weak relationship was observed between peduncle length and severity of FEB with only 6 % of variation being accounted for.

There were significant associations between morphological characters, including cultivar height with peduncle length ($P<0.001$) and LAI with peduncle length (Appendix 4). This suggests that there was a tendency for shorter cultivars, such as Haven, and

dense canopied cultivars such as Apollo, to have shorter peduncles. However, despite these relationships there was substantial variation that could not be explained by the regression formula.

Combining morphological characters of wheat could account for a higher proportion of disease severity than individual characters in these 17 cultivars of wheat. Thus combining the two independent variables of straw height and compactness of ear could account for 57 % of variation according to the multiple regression formula below where:

a = straw height, b = compactness of ear:

$$Y = 152.7 - 0.75a - 239.8b\ x$$

Table 5.1 Mean straw height (cm), peduncle length (mm), leaf area index (LAI) and compactness of ear (number of spikelets/cm rachis) and disease severity in 17 cultivars of winter wheat. Disease severity was assessed as % spikelets showing necrosis 5 weeks after inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65. Cultivars are listed in descending order of height.

Cultivar	Straw height (cm)	Peduncle length (mm)	Leaf area index	Compactness of ear	% spikelets showing necrosis*
Kraka	108.2	194.6	2.2	2.5	12.0
Cadenza	95.4	142.4	1.6	2.4	16.4
Spark	92.2	122.2	2.6	3.0	11.1
Mercia	89.2	132.9	3.1	2.7	24.6
Avalon	86.4	127.9	4.0	2.1	43.5
Apollo	86.3	74.0	3.4	2.4	21.5
Admiral	85.1	110.1	2.8	2.1	36.1
Riband	82.5	107.8	3.4	2.2	34.5
Genesis	80.4	99.1	2.9	2.8	31.8
Hussar	79.8	129.7	2.7	2.4	29.9
Soissons	79.1	116.8	2.1	2.6	23.8
Hunter	79.0	114.9	4.0	2.5	31.6
Haven	78.7	92.3	4.5	2.5	41.8
Hereward	78.6	110.4	3.6	2.7	24.7
Virtue	77.4	142.4	2.9	2.3	45.3
Beaver	76.3	83.4	3.4	2.7	33.7
Brigadier	76.0	125.4	3.6	2.5	35.0
s.e.d ($P<0.05$), d.f. 36	3.0	10.0	0.9	0.9	5.0

* Data angular transformed

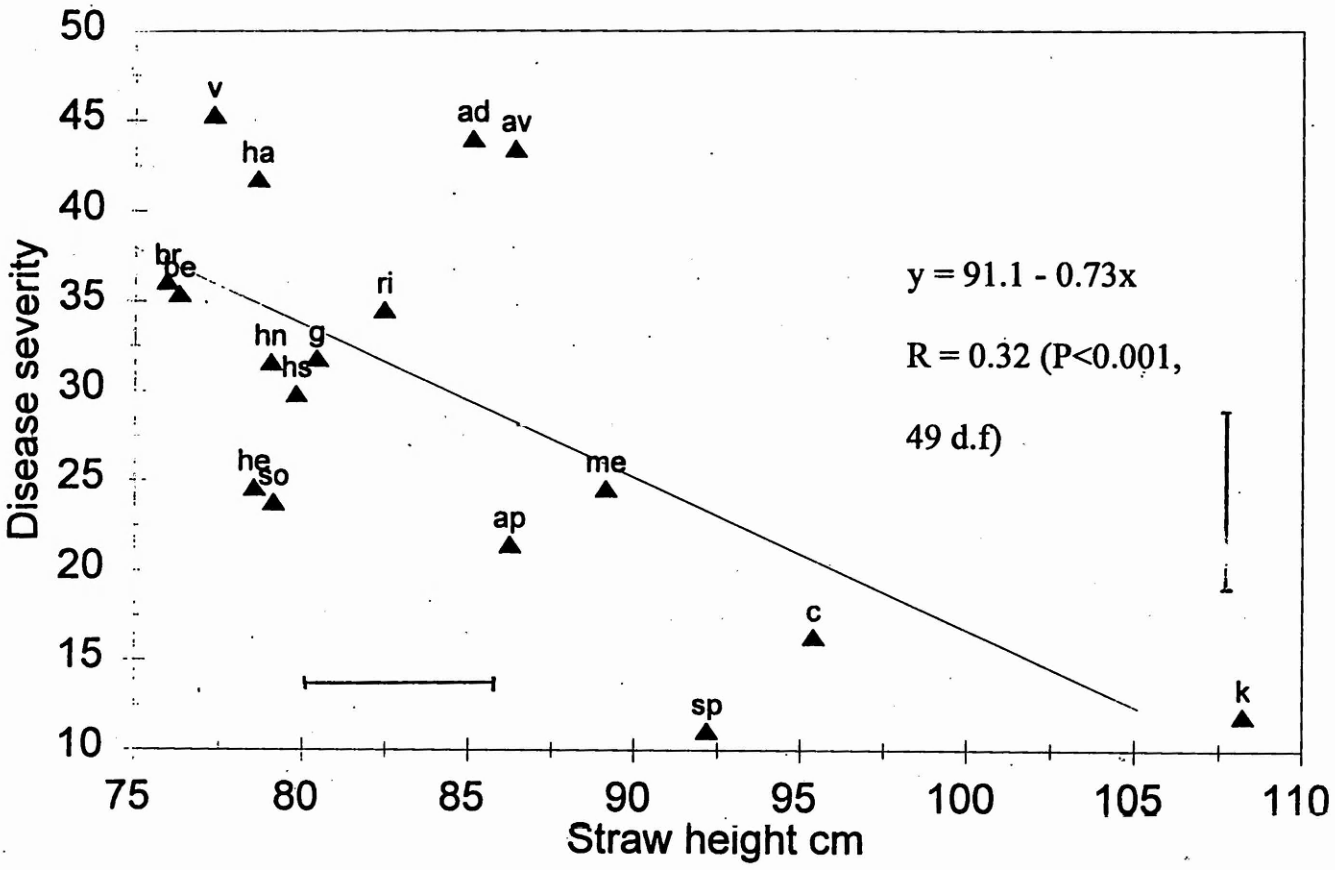


Figure 5.1 Relationship between severity of FEB and mean shoot height of 17 cultivars of winter wheat following inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65. Disease severity was assessed as % spikelets showing necrosis 5 weeks after inoculation and analysed using angular transformed data; bars represent LSD 5% values between cultivars. Key - ad-Admiral, ap-Apollo, av-Avalon, be-Beaver, br-Brigadier, c-Cadenza, g-Genesis, ha-Haven, he-Hereward, hn-Hunter, hs-Hussar, k-Kraka, m-Mercia, r-Riband, so-Soissons, sp-Spark, v-Virtue.

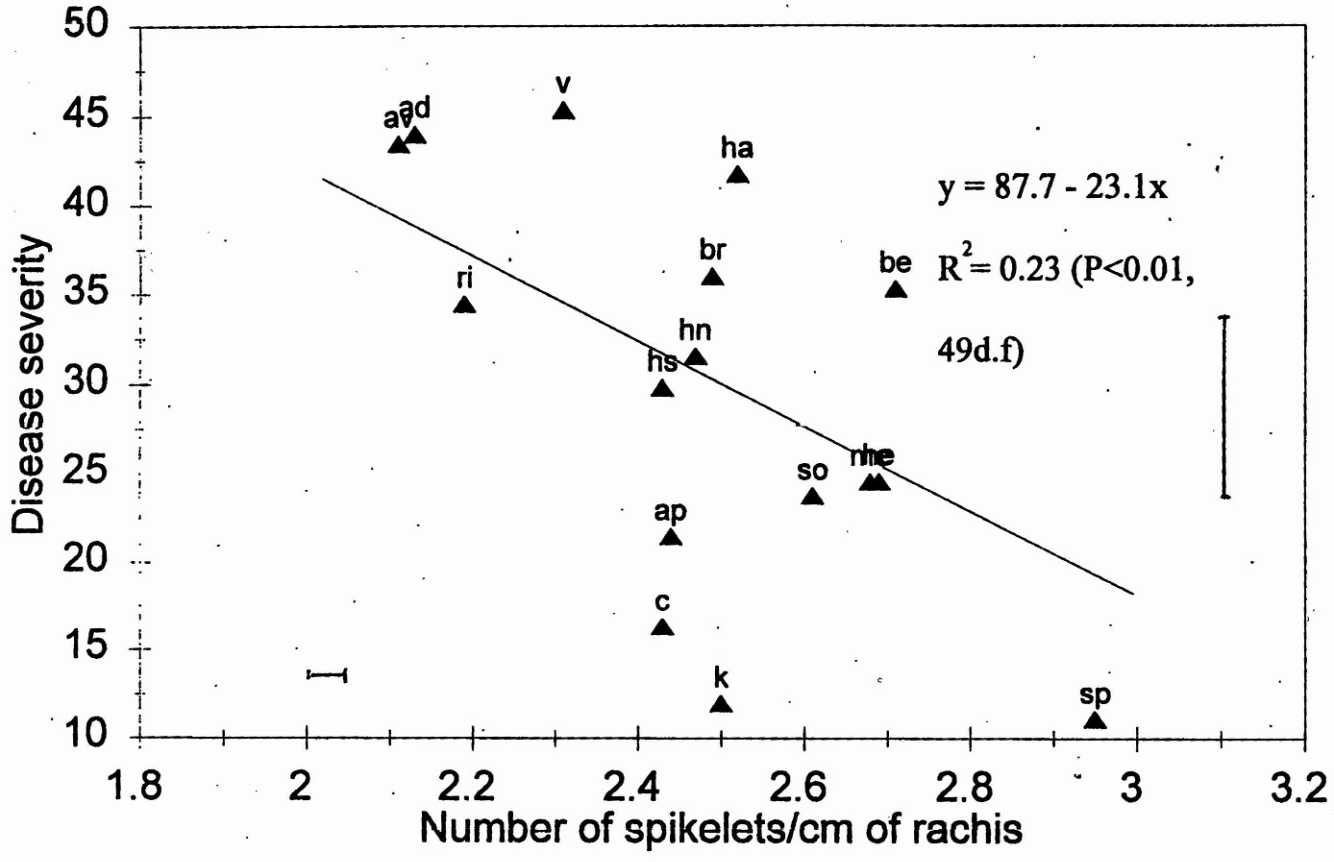


Figure 5.2 Relationship between severity of FEB and mean compactness of ear (number of spikelets/cm of rachis) in 17 cultivars of winter wheat following inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65. Disease severity was assessed as % spikelets showing necrosis 5 weeks after inoculation and analysed using angular transformed data; bars represent LSD 5% values between cultivars. Key - ad-Admiral, ap-Apollo, av-Avalon, be-Beaver, br-Brigadier, c-Cadenza, g-Genesis, ha-Haven, he-Hereward, hn-Hunter, hs-Hussar, k-Kraka, m-Mercia, r-Riband, so-Soissons, sp-Spark, v-Virtue.

5.3.2 Experiment 2 - Analysis of segregating populations

Regression analysis revealed no significant relationship between disease severity and plant height in the F_2's of each cross. However, significant ($P<0.001$) relationships between disease severity and height were identified among the F_3 families of both crosses with taller lines showing less symptoms of FEB than shorter lines. Significant differences ($P<0.001$) were observed between the F_3 families of both crosses in plot height and disease severity. In cross 1, plot height for the F_3 families was distributed between the short parents Encore (85 cm) and Konsul (84 cm) and the tall parent Kraka (109 cm), whilst disease severity was also continuously distributed between Encore and Konsul (63 and 77 %) and Kraka (25 %) (Figure. 5.3). In cross 2, plot height and disease severity for the F_3 families was distributed between the short parent Charger (76 cm) and the tall parent Kraka (113 cm), whilst disease severity was also continuously distributed between Charger (73 %) and Kraka (13 %)(Figure 5.4).

Despite cultivar height accounting for 67 and 70 % of variance in disease severity in amongst F_3 families and parents in cross 1 and 2, respectively, there were significant differences in disease severity between lines of similar height. For example, in cross 1, families of approximately 95 cm in height had significantly different disease severity ranging from 28-58 % spikelets showing necrosis. In cross 2, families at approximately 90 cm in height had significantly different disease severity ranging from 30-62% spikelet necrosis. No relationship between anthesis date, genotype height, and disease severity were established for either cross.

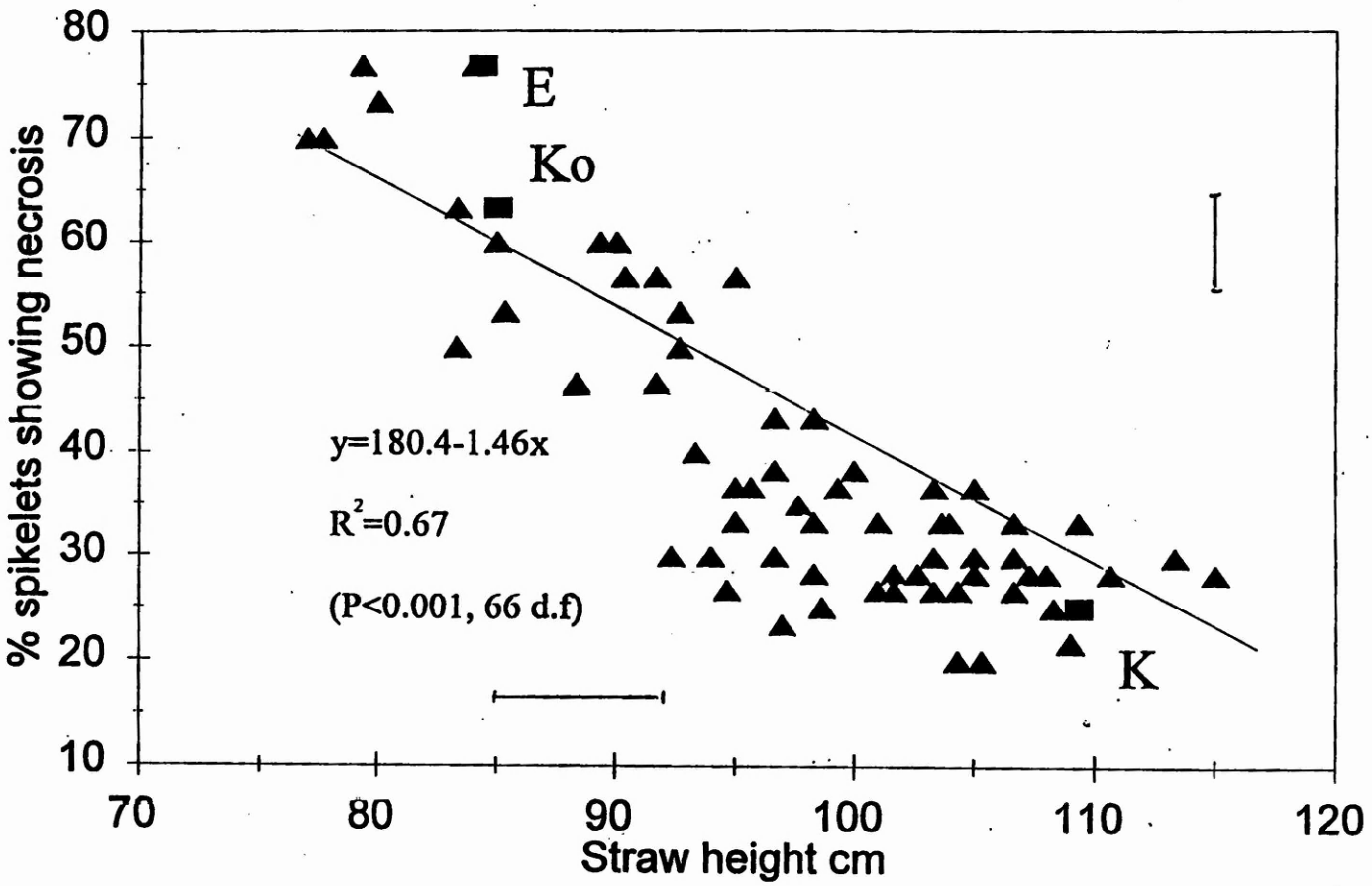


Figure 5.3 Relationship between severity of FEB and straw height in 67 F_3 families and their parents (Kraka x (Encore x Konsul)) following artificial inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65. Percentage of spikelets showing necrosis was measured 5 weeks after inoculation; bars represent LSD 5% values between families. Key- K-Kraka, E-Encore, Ko-Konsul.

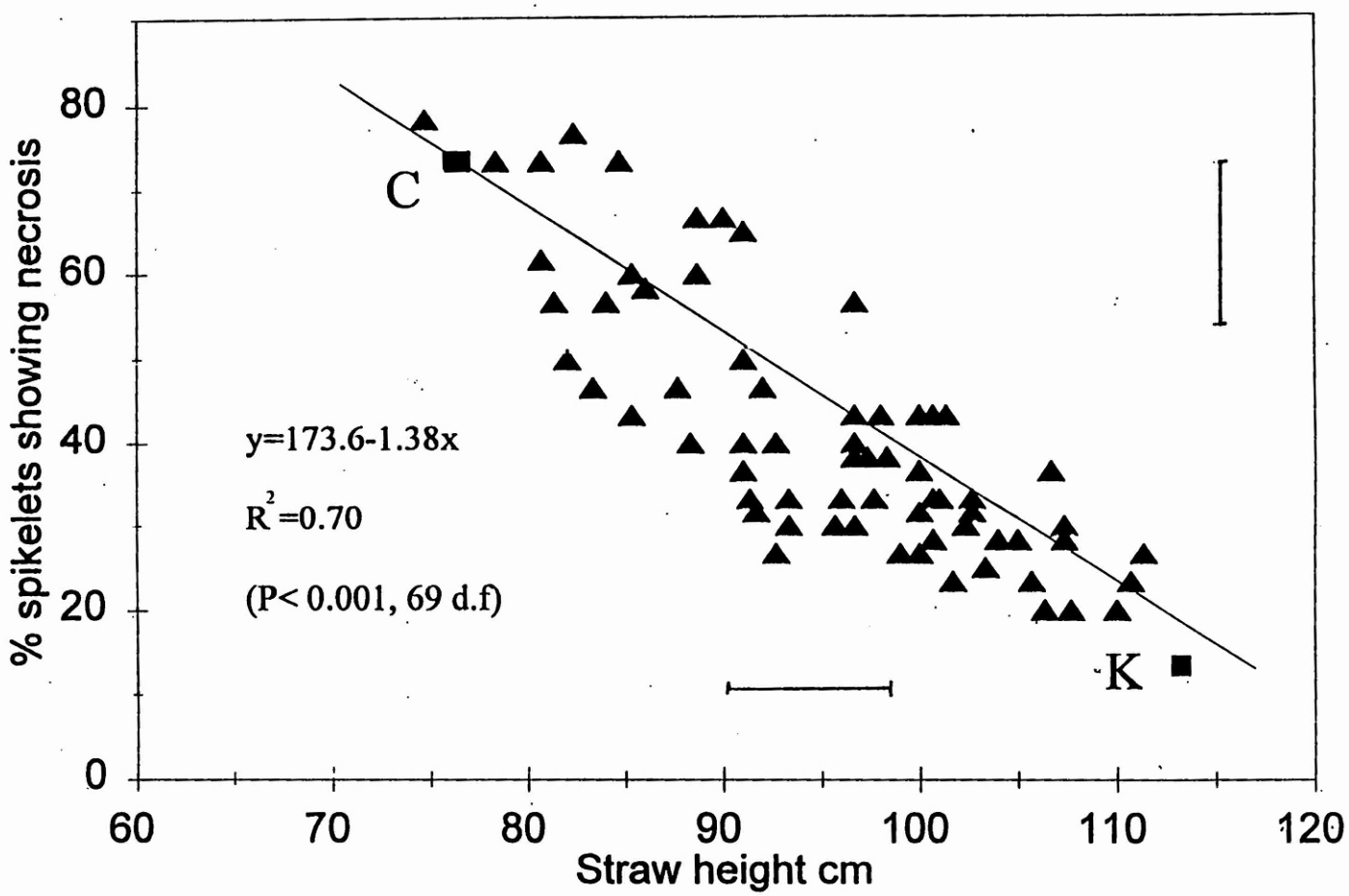


Figure 5.4 Relationship between severity of FEB and straw height in 69 F_3 families and their parents (Kraka x Charger) following inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65. Percentage of spikelets showing necrosis was measured 5 weeks after inoculation; bars represent LSD 5% values between families. Key- C-Charger, K-Kraka.

5.3.3 Experiment 3 and 4 - near isogenic lines of Maris Huntsman and Maris Widgeon differing in the allelic form of the dwarfing genes *Rht1* and *Rht2*

The presence of the dwarfing gene *Rht1* in both cultivars significantly ($P<0.001$) reduced straw height resulting in significant ($P<0.001$) increases in disease severity. For example, lines of Maris Huntsman containing the *Rht1* allele showed 81 % of spikelets showing necrosis whilst those with the *rht1* allele showed 39 % (Table 5.2). Shorter lines of Maris Widgeon also had more disease than taller ones, although data was not always statistically significant. Reductions in height due to the presence of *Rht1* or *Rht2* in Maris Huntsman during 1996/97 were also associated with significant ($P<0.001$) increases in disease severity (Table 5.3). No differences in disease severity or straw height were observed between those lines containing either the *Rht1* or *Rht2* dwarfing genes.

Table 5.2 Straw height and severity of FEB in near-isogenic lines of Maris Widgeon and Maris Huntsman with and without the dwarfing gene *Rht1*. Percentage of spikelets showing necrosis was recorded 5 weeks after inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65.

	Background	Maris Huntsman		Maris Widgeon	
		straw height (cm)	% spikelets showing necrosis	straw height (cm)	% spikelets showing necrosis
Rht1	a	91.8b	78.7a	100.0c	37.5ab
Rht1	b	90.3b	83.7a	106.5b	47.5a
rht1	a	107.8a	40.0b	116.8a	27.5b
rht1	b	107.5a	37.5b	115.5a	33.7b
s.e.d. ($P<0.05$), d.f .9		1.1	7.6	2.1	4.1

Numbers within a column followed by the same letter are not significantly different according to Duncans Multiple range test.

Table 5.3 Straw height and severity of FEB in near-isogenic lines of Maris Huntsman with and without the dwarfing genes *Rht1* and *Rht2*. Percentage of spikelets showing necrosis was measured 5 weeks after inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65.

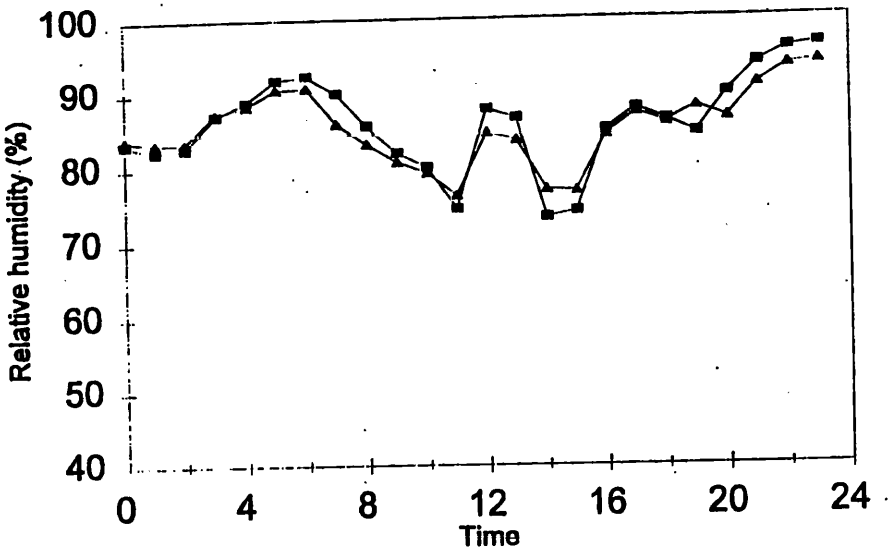
	Background	straw height (cm)	% spikelets showing necrosis
Rht1	a	62.9c	43.8abc
	b	63.1c	47.8ab
rht1	a	81.3a	30.3bcd
	b	81.1a	29.3cd
Rht2	a	61.5c	39.2abc
	b	60.7c	49.3a
rht2	a	82.3a	26.3cd
	b	76.3ab	18.0d
s.e.d. (P<0.05), d.f. 21		1.5	4.9

For each cultivar numbers in each column followed by different letters are significantly different (P=0.05), according to the Duncans Multiple range test.

5.3.4 Experiment 5 - Measurement of humidity at ear height

Humidity throughout the day generally followed a diurnal pattern from over 90 % during the night, down to 60 % at 1500 hours. This pattern was altered by environmental conditions such as rainfall. For example, June 28, had particularly high humidity which was associated with 7.9 mm of rain on that day (Figure 5.5a), whilst on June 29 there was no rain (Figure 5.5b). Analysis of variance of hourly readings and area under the humidity progress curve showed that there was no significant differences in humidity between the two lines of Maris Huntsman at any time between 27 June and 30 July 1997 (mean data presented in Appendix 5). No significant differences were observed between the two lines of Maris Huntsman in LAI.

(a)



(b)

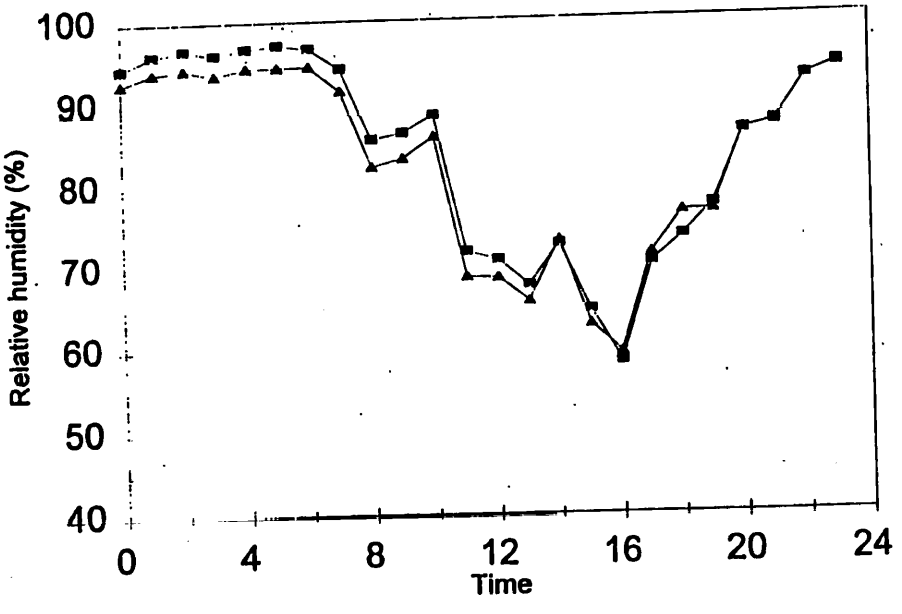
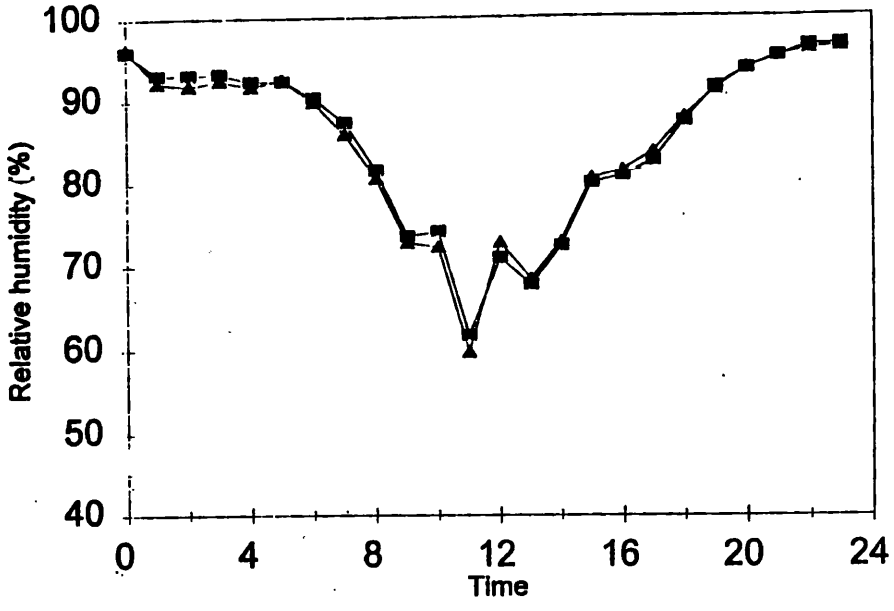


Figure 5.5 Relative humidity at ear height in isogenic lines of Maris Huntsman without (■ ; normal) and with (▲ ; short) the dwarfing gene *Rht1* on June 28 (a) and June 29 (b). Each line represents the mean relative humidity recorded by four sensors set up at ear height and is expressed at hourly intervals throughout the day (0 = midnight).

(c)



(d)

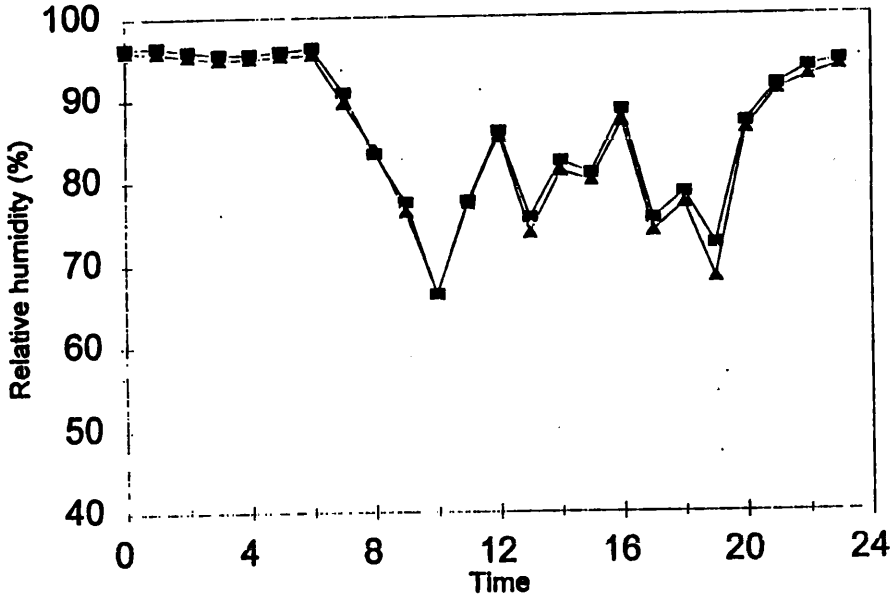


Figure 5.5 Relative humidity at ear height in isogenic lines of Maris Huntsman without (■ ; normal) and with (▲ ; short) the dwarfing gene *Rht1* on June 30th (c) and July 1st (d). Each line represents the mean relative humidity recorded by four sensors set up at ear height and is expressed at hourly intervals throughout the day (0 = midnight).

5.4 Discussion

5.4.1 Relationship between straw height and severity of FEB

In naturally infected field trials in 1984 and 1987 involving over 400 genotypes, Mesterhazy (1995) showed that taller genotypes of winter wheat showed a reduced severity of FEB compared with shorter ones. It was concluded that this was due to simple disease escape, with taller genotypes having ears further from the sources of inoculum such as crop debris at the base of the crop. However, results obtained from studies reported here, with artificial inoculation, showed that there was a linear relationship between straw height and severity of FEB, with tall genotypes showing reduced symptoms. Although the relationship between cultivar height and severity of FEB was weak, particularly when the outlying cultivar Kraka (108cm) was removed from the regression equation (Figure 5.1), straw height only ranged from 76-95cm. However, a higher proportion of variance was accounted for amongst F_3 families where straw heights were normally distributed between 75-115 cm (Figures 5.3 and 5.4). In these studies, as control plots were relatively free from disease, natural infection was considered to be relatively unimportant in these studies and hence the basis of this relationship was believed to be caused by factors other than simple disease escape.

This present study also shows that the severity of FEB in winter wheat is likely to be a quantitative character controlled by a number of genes. Evidence from F_3 families, derived from crosses between short and tall parents of winter wheat, showed that lines of similar height could differ significantly in the % spikelets showing necrosis (Figure 5.3 and 5.4), suggesting that disease severity in individual genotypes is not only affected by its straw height but also by other genes independent of height. Other workers have

also suggested that resistance to FEB is a quantitatively controlled character controlled by a number of genes (Snijders 1990d; Singh *et al.*, 1995; van Ginkel *et al.*, 1996). Continuous variation in straw height among F_3 families was also observed in our study despite the presence of the dwarfing gene *Rht2* in the susceptible / short-strawed parent Charger found in cross 2 of this study. It would appear that the effect of these dwarfing genes was masked by the effect of modifier genes. Gale and Law (1977) showed that when the dwarfing gene *Rht2* was segregating in crosses involving Maris Fundin continuous variation in height was also observed.

The clear tendency for tall straw and resistance to FEB to co-segregate among F_3 families suggests that this relationship has a genetic basis that could either be explained by linkage, between one or more genes controlling resistance to FEB and genes controlling straw height, or pleiotropy, where genes that promote shorter straw also promote susceptibility to FEB. If pleiotropy is involved, there must be mechanisms such as increased relative humidity at ear height in shorter genotypes of wheat, which could account for increased severity of FEB. In this work there was no difference in relative humidity at ear height between tall and short isogenic lines of Maris Huntsman. However, it is possible that differences in ear surface wetness (dew) may exist that could explain a pleiotropic response. Dew typically forms on clear nights when the surface temperature falls below the temperature at which air becomes saturated with water vapour, so that any further cooling results in condensation of liquid water. Thus dew will form on any exposed surface including wheat ears. A meterological study of wheat crops found that the period saturation on a dewy night was shorter for a less dense stand of wheat (Penman and Long, 1960). Future experiments could include measuring

duraton of leaf surface wetness with a leaf wetness metre or weight of surface water. Measuring dew weight can be achieved on dewy summer mornings using a method adapted from Scott *et al.* (1985) by applying pre-weighed discs of filter paper to ears to absorb available moisture and then re-weighing the paper to determine the water content.

Other possible pleiotropic mechanisms could include increased concentration of assimilate in the ears of short cultivars that will encourage infection by *Fusarium* spp and *M. nivale* in these genotypes. Austin *et al.* (1980), who compared a range of modern wheats with cultivars released during the previous 70 years, showed a 44 % yield increase during 50 years of breeding and concluded this was due to an increase of harvest index from 0.35 to 0.50, as a result of selection of shorter cultivars. They believed that a higher harvest index corresponded to increased partitioning of assimilates to the developing ear.

The presence of the dwarfing genes *Rht1* and *Rht2* was associated with increased severity of FEB in near-isogenic lines of Maris Huntsman and Maris Widgeon. Linkage between the *Rht2* dwarfing gene and resistance to the mainly foliar pathogen of wheat *Septoria tritici* was suggested by Baltazar *et al.* (1990), who showed that isogenic lines of wheat containing the *Rht1* gene were more susceptible to disease than those with the *Rht2* gene. In the present study, there was no indication that isogenic lines of Maris Huntsman or Maris Widgeon containing *Rht1* had more severe symptoms of FEB than those containing *Rht2*. However, as both straw height and severity of FEB are controlled by a number of genes, it is difficult to postulate numerous linkages between these

characters. Despite this, linkage appears the most likely explanation at present to account for the relationship between severity of FEB and straw height in winter wheat.

Scott *et al.* (1982) found a similar negative relationship between disease severity and plant height in winter wheat cultivars following artificial inoculation with the foliar and ear pathogen of wheat, *Septoria nodorum*. By using random F_3 and F_4 derived lines from five crosses between cultivars of varying height, this relationship was also explained either by linkage or by a pleiotropic effect. One possible hypothesis for this pleiotropic association was that shorter cultivars provide denser leaf canopies and, as such, produce a more favourable microclimate for the development of *S. nodorum* (Scott *et al.*, 1985). By measuring leaf surface wetness, using electrical resistance between two copper wires, and estimating dew on the flag leaf by increases in weight of small pieces of filter paper, it was shown that leaf wetness (mainly dew) lasted for a longer time on short cultivars than on tall ones. However, this microclimate effect was believed to be one of several mechanisms that contributed to shorter cultivars having more severe symptoms of *S. nodorum* (Scott *et al.*, 1985).

This study has highlighted the existence of lines with different amounts of disease within a particular height class. At the 90 - 95 cm height, incidence of FEB in lines varied from about 25 to 65 % (Figures 5.3 and 5.4) suggesting that there are additional genes affecting resistance independent of height which could be utilised by plant breeders. Scott *et al.* (1982) also concluded that there were genes controlling resistance to *S. nodorum* independent of straw height and suggested that it would be possible to breed moderately-resistant cultivars of any height, but with increasing difficulty as straw

height is reduced. The recent production of the cultivar Charger which is short strawed and has moderate resistance to *S. nodorum* (Anon., 1997) is evidence of this. In the same way it should be possible to select short strawed genotypes with moderate resistance to FEB, although this would become increasingly difficult with a reduction in straw height because of reduced variation in disease severity and a smaller population size from which to select such genotypes.

This study has clearly indicated that the relationship between severity of FEB and straw height in wheat has a true genetic basis. However, further work, is required to identify whether this relationship is due to linkage, pleiotropy or a combination of both these mechanisms.

5.4.2 Relationship between cultivar morphology and severity of FEB

Observations made by Mesterhazy (1995) suggested that wheat cultivars with compact ears were more susceptible to FEB than open ones in a naturally infected field trial. However, results reported in this artificially inoculated field study, showed that open eared cultivars, such as Avalon, were more susceptible to FEB than cultivars with compact ears, such as Spark. Even when the outlying cultivar Spark (Figure 5.2) was removed from the regression equation this relationship was still found to be significant. Why cultivars with an open ear should be more susceptible to FEB following artificial inoculation is unclear. Liang *et al.* (1981) observed that the most susceptible winter wheat cultivars retained anthers inside the glumes. Observations by the author in the glasshouse and the field at Harper Adams suggest that the susceptible cultivar Avalon also maintains its anthers within the glumes, which potentially, allows more inoculum

to be retained on the ear following artificial inoculation by *Fusarium* spp. It is thus possible that open eared cultivars may retain more inoculum on the ear following artificial inoculation.

A weak relationship between density of leaf canopy and severity of FEB in 17 cultivars of winter wheat was also observed, with denser leaf canopied cultivars, such as Haven, showing more symptoms of FEB. Tompkins *et al.* (1993) found that denser plots of winter wheat favoured the development of *S. nodorum* by creating a more favourable microclimate. Plots that had been sown at narrow row spacings (9 cm) and which received high levels of nitrogen fertiliser (140 kg/ha) showed increased leaf wetness compared with plots which had wide row spacing (36 cm) and low levels of nitrogen (35 kg/ha). A weak but significant relationship was also noted between peduncle length and disease severity, with those cultivars having shorter peduncles showing more severe symptoms of FEB. However, the significant relationship between peduncle length and straw height would suggest that any effect on disease severity is due to cultivar stature. As only 11 and 6 % of variance in severity of FEB could be accounted for by LAI and peduncle length, respectively, they are not considered to be important factors in determining resistance among different cultivars of wheat in the field.

By combining the two independent variables of straw height and compactness of ear, 53 % of variance in disease severity could be accounted for. It is, therefore, proposed that assessing genotypes for these characters could be a useful tool in producing cultivars with improved resistance to FEB. Indeed, Mesterhazy (1995) has described an ideotype of wheat that would give limited control of FEB under conditions of natural

infection. He suggests "The optimal plant structure involves plant height about 90 - 100 cm, the plant should be awnless and the distance between flag leaf and ear should be at least 15 cm, whilst the head should not be too dense". However, further work in producing a model to select genotypes resistant to FEB on the basis of morphological characters has to be performed before breeding towards an ideotype can begin.

CHAPTER 6

General Discussion

Recent epidemics of FEB in wheat around the world have increased concern about *Fusarium* mycotoxins and yield losses associated with the disease. In Minnesota during 1993, wheat and barley producers bore losses of 1 billion dollars, with the damage equivalent to a 33% decrease in wheat production (Dill-Macky, 1997). In China, serious epidemics have occurred in 7 years between the period 1951-1985 (Zhuping, 1994). Controlling this disease has proved difficult. Fungicide control is at best inconsistent (Milus and Parsons, 1994) due to poor active ingredients (Parry *et al.*, 1995), application timing (Hutcheon and Jordan, 1992) and the detrimental effect of these compounds on beneficial microflora that naturally reduce levels of *Fusarium* infection on wheat ears (Liggitt *et al.*, 1997). Thus, producing cultivars with resistance to FEB appears to be the best option in controlling this disease. Although a number of genotypes have been identified with good resistance to FEB (Snijders, 1990a; Liu and Wang, 1991; Mesterhazy, 1995) most have agronomically unsuitable characters. A fuller understanding of the mechanisms of resistance to FEB will lead to more efficient procedures to identify and screen suitable genotypes.

6.1 Components of resistance

A simplified model of FEB resistance was proposed by Schroeder and Christensen (1963), who suggested that resistance was of two types; resistance to initial infection (Type I) and resistance to the spread of infection within the ear (Type II). As anthesis is the critical period for infection by *Fusarium* spp (Atanasoff, 1920; Strange and Smith, 1971) it is proposed that the basis of Type I resistance in wheat is due to differences between cultivars in the flowering structure. Several authors have related flower morphology with resistance to initial infection. For example, Liang *et al.* (1981)

observed that the most susceptible cultivars held their anthers inside the glume. Earlier work indicated that the position of the dehisced anthers within the glumes themselves was also critical to resistance to initial infection by *F. graminearum* (Takegami, 1957b). The author placed cultivars into distinct groups according to flower morphology before comparing initial infection in these cultivars. Group A, consisted of cultivars that caught their anthers between the tips of the closing glumes and were thus susceptible from an early stage of flowering. Whilst, Group B, did not expose its anthers until a later stage of flowering, due to being trapped behind the folded margin of the paleae. Thus these cultivars were susceptible to infection at a later stage of flowering (Takegami, 1957b).

It has also been proposed that anthers from particular cultivars may also encourage initial infection. It was shown in Chapter 3 that anthers from the cultivars Mercia and Beaver caused significantly more germ-tube branching of conidia of *F. culmorum* compared with Riband and Hussar. It was suggested that an increase in the number of germ-tubes could be regarded as an increase in the number of potential infection sites. Thus, Mercia and Beaver were believed to be more susceptible to initial infection than Hussar and Riband. Why anthers from some cultivars stimulates germ-tube branching more than others is unclear. Whether such differences are due to varying concentrations of choline and betaine (Strange and Smith, 1978), reducing sugars or amino acids is not known and therefore further studies into the biochemical basis of type I resistance is required.

Previously, workers have measured Type I resistance on different cultivars by spraying conidial suspension onto the ears of winter wheat and then assessing the number of

infected spikelets (Schroeder and Christensen, 1963; Saur, 1984) or by injecting inoculum into a number of individual spikelets on each head and then recording the percentage of infected spikelets (Yong-Fang *et al.*, 1997). However, these methods rely on recording infection or visual symptoms which will not only be due to resistance to initial infection (Type I) but also to colonisation (Type II) within the spikelet itself. Once the stimulatory compounds in the anthers in wheat are identified, it may be possible to select genotypes for increased Type I resistance according to their biochemical make up. However, the best method at present may be the *in vitro* technique devised in Chapter 3 which tests anthers from different cultivars of wheat for their ability to stimulate germ-tube branching in conidia of *Fusarium* spp.

Use of the point inoculation method adopted in Chapter 4 showed that twelve cultivars of winter wheat differed in resistance to colonisation (Type II) of the ear, following inoculation with *F. culmorum*, confirming the findings made by other workers (Saur and Morlais, 1984; Yong-Fang *et al.*, 1997). In severe cases, a secondary symptom of premature bleaching (scalding) related to high % necrosis was observed above the point of infection. Indeed, cultivars differed significantly in severity of scalding following inoculation with both *F. culmorum* and *F. graminearum*. Although symptoms of necrosis and scalding may visually appear similar they are in fact both distinct, with necrosis being related to infection by the pathogen, whilst scalding is a secondary symptom associated with severe necrosis. Therefore, when assessing for resistance to colonisation (Type II) among different cultivars of wheat, care must be taken not to confuse these symptoms and overestimate disease severity because of scalded spikelets.

Although point inoculation of individual spikelets within the middle of the ear allowed a detailed study of symptom development, it did not reveal why certain cultivars were more resistant to fungal colonisation (Type II) or scalding. Further work is, therefore, required to understand whether the reduced spread of infection within the ears of resistant cultivars is due to degradation of the mycotoxin DON (Type III resistance) (Millar and Arnison, 1986), or the ability of some cultivars to tolerate high DON concentrations (Type IV resistance) (Wang and Miller, 1988) or a reduced size of vascular bundles in resistant cultivars that could restrict fungal colonisation. Similarly, the mechanisms behind scalding are not understood, so a histological study looking at cross sections from infected rachis segments could reveal whether this symptom is the result of the fungus blocking the vascular bundles in the rachis and hence cutting off the water and food supply above the point of infection as proposed by Atanasoff (1920).

As no relationship between stimulation of germ-tube branching (Chapter 3) and resistance to colonisation (Chapter 4) was observed, the simple model proposed by Schroeder and Christensen (1963) with two types of resistance working independently of each other appears to be appropriate. It was, therefore, concluded that symptoms of FEB seen on different cultivars of wheat are not only due to differences in initial infection (Type I) and spread of necrosis (Type II) but also differences in scalding. Thus when selecting genotypes of wheat, breeders could choose lines with either individual or both types of resistance.

6.2 Passive resistance

Associated with the two genetic components of resistance (Type I and II) it is proposed

that certain morphological characters also affect severity of FEB. In Chapter 5 it was shown that cultivar height, in particular, was related to the percentage of spikelets showing necrosis following artificial inoculation. Thus, short cultivars of wheat, such as Virtue, showed more symptoms of FEB than taller cultivars such as Kraka.

The relationship between disease severity and straw height was shown to have a genetic basis which could either be explained by linkage, between one or more genes controlling resistance to FEB and genes controlling straw height, or pleiotropy, where genes that promote shorter straw also promote susceptibility to FEB. It was speculated that one possible mechanism for pleiotropy is increased humidity at ear height among shorter cultivars. However, in this work no significant differences in relative humidity at ear height were observed in a short and tall isogenic line of Maris Huntsman. If pleiotropy is involved there must be other mechanisms such as increased surface wetness or assimilate in the ears of shorter cultivars that would encourage infection by *Fusarium* spp and *M. nivale.*

Although straw height accounted for 70 % of variation in disease severity among F_3 families, derived from crosses between tall and short cultivars of wheat, considerable variation was unexplained. For example, at the 90 - 95 cm height class, disease severity on lines varied from 25 to 65 % suggesting that there were additional genes independent of height which could affect resistance. Thus by utilising genes independent of height it will be possible to produce a cultivar of any height with moderate resistance to FEB. A similar relationship between cultivar height in wheat and disease severity was also observed following artificial inoculation with *S. nodorum* (Scott *et al.*, 1982; 1985) and

useful comparisons between these two diseases in this respect can be made.

6.3 Future work

This work has revealed that the mechanisms of resistance to FEB in wheat are complex. Symptoms of FEB in the field are not only the result of resistance to initial infection, spread of infection and scalding but also a number of morphological characters of wheat cultivars. Suggested further work includes:

a) Further investigation on the effect of anthers from different cultivars of wheat on infection by *Fusarium* species. In particular, the analysis of reducing sugars, amino acids, choline and betaine concentrations in anthers from different cultivars may reveal if Type I resistance has a biochemical basis.

b) Histological investigations to study differences in the vascular tissue, from a range of cultivars of wheat, which could be related to Type II resistance. For example, do resistant cultivars have wider xylem vessels, that are less likely to become blocked with mycelium, making them less likely to show symptoms of scalding?

c) Inoculating individual spikelets from a range of cultivars with either a tricothecene producing or a non-mycotoxin producing *Fusarium* isolate to study the effect of these toxins on spread of infection. Comparing disease severity in these ears would reveal information on the importance of DON in terms of pathogenicity and reveal wether resistant cultivars can tolerate higher concentrations of DON compared with susceptible ones.

d) Further investigation on whether the relationship between cultivar height and severity to FEB is due to 'linkage' or 'pleiotropy'. Linkage or pleiotropy could be assessed by examining specific generations from crosses between short susceptible and tall resistant winter wheat cultivars. In the absence of linkage genetic variance between generations will be similar, whilst differences between generations would imply linkage. A similar study by Law *et al.* (1978) compared the total covariance of F_2, F_2 X F_1 and F_2 X F_2 generations to show that both linkage and pleiotropy were involved in the positive correlation found between height and yield in wheat. Linkage could also be assessed using quantitative trait loci (QTLs) to map genes affecting straw height and resistance to FEB. Molecular markers used could include RFLPs, RAPDs, AFLPs or even microsatellites and simple sequence repeats (SSRs). One possible hypothesis for a pleiotropic association is that shorter cultivars have a more favourable microclimate at ear height for the development of *Fusarium* spp. By comparing disease severity in short and tall genotypes grown under controlled environmental conditions following artificial inoculation could reveal whether there is a basis to this idea.

e) Further investigation on the relationship between morphological characters, including straw height and compactness of ear of different cultivars of wheat. A model from such a study could be used to indicate an 'ideotype' which would be resistant to FEB.

6.4 Conclusions

1) Anthers from cultivars of winter wheat stimulated incidence of germination, germ-tube branching and length in conidia of *F. culmorum*. Anthers from certain cultivars also encouraged more germ-tube branching and it is believed this may explain differences in susceptibility to intial infection (Type I resistance).

2) Cultivars differed in spread of infection (Type II resistance) following inoculation of a single spikelet in the middle of the ear with *F. culmorum* or *F. graminearum*. Two distinct symptoms were observed: necrotic spikelets below the point of inoculation which were caused by infection and premature bleaching (scalding) above the point of inoculation which was associated with cultivars showing high levels of necrosis.

3) Certain morphological characters including straw height and compactness of ear affect severity of FEB. Thus short strawed cultivars with lax ears had more symptoms of FEB than taller strawed cultivars with dense ears.

4) The relationship between disease severity and straw height has a genetic basis which is believed to be due to linkage or pleiotropy. Monitoring of relative humidity at ear height in a short and tall isogenic line of Maris Huntsman revealed no significant differences between these genotypes suggesting that microclimate cannot explain increased severity of FEB in shorter strawed cultivars of wheat. It is suggested that there are independent genes affecting severity of FEB that will allow selection of cultivars of any height.

5) Symptoms of FEB in the field are not only the result of resistance to initial infection, spread of infection and scalding but also a number of morphological characters of wheat cultivars.

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APPENDICES

Appendix 1. Relationship between the percentage of necrotic spikelets in the proximal section and the proportion of scalding in the distal section of individual ears from 12 cultivars of winter wheat following inoculation with *F. culmorum*. Spikelet ten (counting from the base of the ear) was inoculated at mid-anthesis and symptoms were recorded 4 weeks later. Regression analysis using a binomial logistic model was fitted, with the total number of spikelets in the distal region as the binomial totals and the number of scalded spikelets as the response variate. The relationships are expressed as individual cultivar regression equations with standard errors.

Cultivar	Y intercept	Slope, b	Standard error
Admiral	-12.3	0.048	14.4
Apollo	-3.3	0.048	14.4
Avalon	-3.7	0.048	14.4
Beaver	-6	0.048	14.4
Brigadier	-4.4	0.048	14.4
Genesis	-3.6	0.048	14.4
Haven	-5	0.048	14.4
Hereward	-3.5	0.048	14.4
Hunter	-3.8	0.048	14.4
Hussar	-3.3	0.048	14.4
Mercia	-13.4	0.048	18.3
Riband	-4.6	0.048	14.4

Appendix 2. Relationship between the percentage of necrotic spikelets in the proximal section and the proportion of scalding in the distal section of individual ears from 12 cultivars of winter wheat following inoculation with *F. graminearum*. Spikelet ten (counting from the base of the ear) was inoculated at mid-anthesis and symptoms were recorded 4 weeks later. Regression analysis using a binomial logistic model was fitted, with the total number of spikelets in the distal region as the binomial totals and the number of scalded spikelets as the response variate. The relationships are expressed as individual cultivar regression equations with standard errors.

Cultivar	Y intercept	Slope, b	Standard error
Admiral	-13	-0.03	1.61
Apollo	-17	0.54	2.69
Avalon	-15	0.26	1.79
Beaver	-14.2	0.02	2.51
Brigadier	-1.6	-0.01	1.61
Genesis	-13	-0.03	13.6
Haven	-3.9	0.02	1.61
Hereward	-4.1	0.04	1.61
Hunter	-5	0.08	1.61
Hussar	-1.7	0	1.61
Mercia	-13.3	-0.01	3.22
Riband	-13.8	0.02	4.43

Appendix 3. Relationship between the number of necrotic spikelets and infection of the spikelets and rachis segments following artificial inoculation of the 10th spikelet from the base with *F. culmorum*. Spikelets were examined for necrosis 4 and 6 weeks after inoculation and then related to the number of infected spikelets and rachis segments which was assessed by re-isolating the fungus onto PDA. The relationships are expressed as R^2 values obtained from regression analysis.

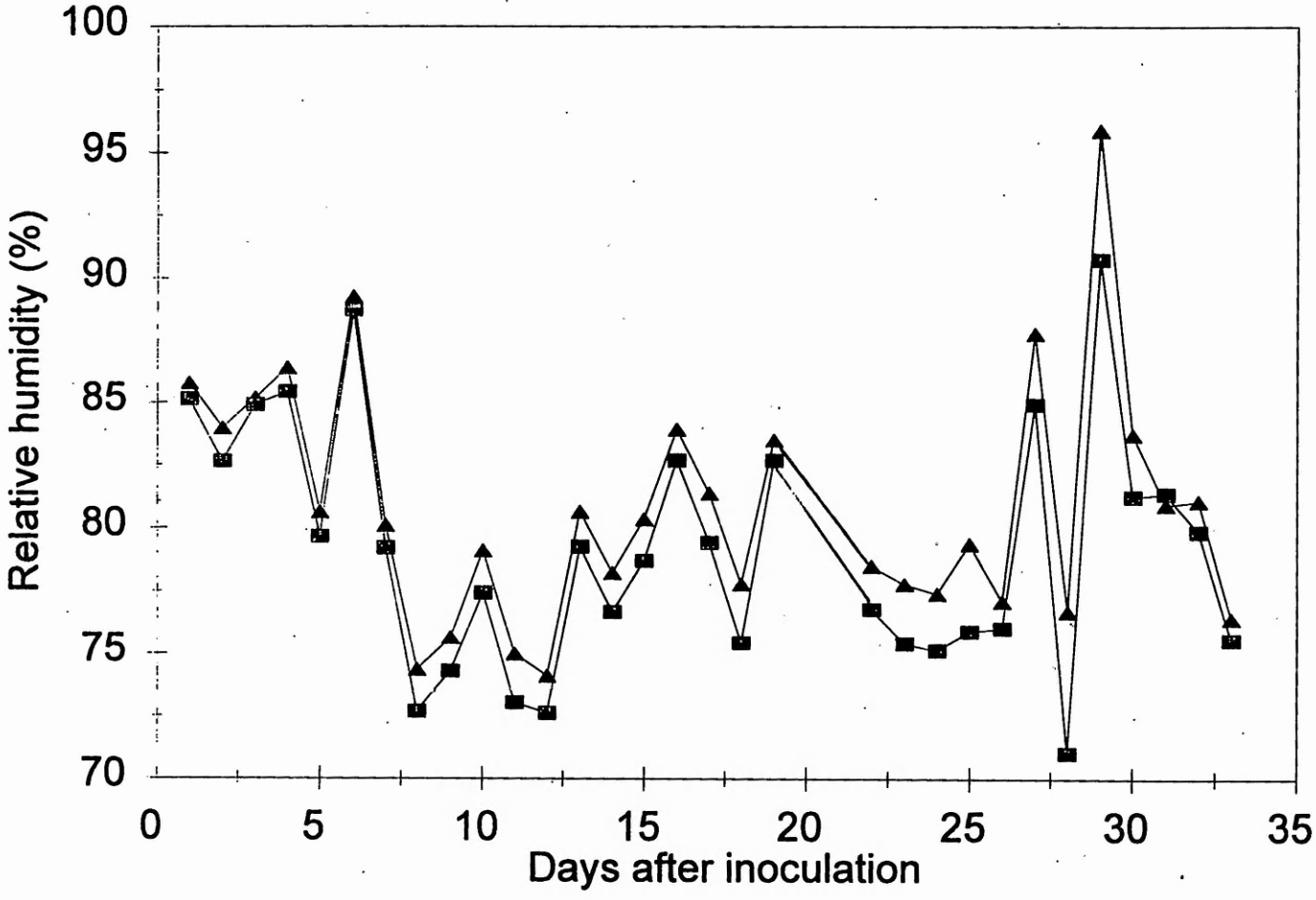
Cultivar	Y intercept	Slope, b	R^2
Relationship between spikelet necrosis and spikelet infection			
Sumai -3	1.0	0	1.0
Ringo star	0.03	1.19	0.9
Riband	0.21	0.84	0.93
Virtue	1.74	0.53	0.52
Kraka	0.1	0.9	1.0
Relationship between spikelet necrosis and rachis infection			
Sumai - 3	*	*	*
Ringo star	0.30	0.54	0.58
Riband	0.59	0.64	0.99
Virtue	1.08	0.78	0.63
Kraka	0.32	0.62	0.88
Relationship between spikelet and rachis infection			
Sumai - 3	*	*	*
Ringo star	0.24	0.45	0.64
Riband	0.58	0.72	0.95
Virtue	0.56	1.12	0.69
Kraka	0.24	0.07	0.88

* Insignificant infection of the rachis in Sumai - 3

Appendix 4. Relationship between straw height, peduncle length, compactness of ear, leaf area index (LAI) and severity of FEB on 17 cultivars of winter wheat. Disease severity was assessed as % spikelets showing necrosis 5 weeks after inoculation with a mixture of *Fusarium* spp and *M. nivale* at GS 65 and statistical analysis was done on angular transformed data.

	Y intercept	Slope, b	R^2
Relationship between morphological characters and disease severity			
Straw height	91.1	-0.73	0.32**
peduncle length	41.9	-0.13	0.08*
LAI	18.1	3.99	0.14*
Compactness of ear	87.7	-23.5	0.23**
Relationship between morphological characters			
Straw height and peduncle length	-53.8	2.06	0.40**
Straw height and LAI	6.31	-0.04	0.08
Straw height and compactness of ear	0.26	0	0
Peduncle length and LAI	5.56	-0.02	0.19*
Peduncle length and compactness of ear	0.26	0	0.02
LAI and compactness of ear	0.26	-0.0	0

** Significant to 1 % level
* Significant to 5 % level



Appendix 5. Mean relative humidity at ear height in isogenic lines of Maris Huntsman without (■ ; normal) and with (▲ ; short) the dwarfing gene *Rht1* between 27th June and 30th July. Each line represents the mean relative humidity of each day recorded by four sensors set up at ear height at hourly intervals throughout the day. Date is expressed as number of days after 27th June.