CRANFIELD UNIVERSITY

ROTHAMSTED RESEARCH

Sarah Holdgate

IMPROVING THE DIVERSITY OF RESISTANCE MECHANISMS AVAILABLE IN WHEAT TO COMBAT FUSARIUM EAR BLIGHT DISEASE

Faculty of Medicine and Biosciences

Ph.D. Thesis

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Supervisors:

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Professor Kim Hammond-Kosack

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Abstract

Fusarium ear blight (FEB) is a disease of wheat and small grain cereals, caused by the fungi *Fusarium culmorum* and *Fusarium graminearum*. The disease causes premature bleaching of spikelets and shrivelling of the grain can result in a direct yield loss. Mycotoxins such as deoxynivalenol produced by the fungus can reduce grain quality. Breeding for resistant wheat cultivars is considered one of the best control options. Previously identified resistance has been reported in the Chinese genotype Sumai 3.

The principal aim of this project was to identify novel sources of resistance to FEB. Twenty four wheat genotypes were evaluated for resistance to FEB in this project. Molecular markers linked to previously identified QTL from Sumai 3 conferring resistance were used to confirm their absence in the genotypes under investigation, and revealed that none of the genotypes under investigation contained all of the QTL for resistance. Field trials conducted over two years screening for resistance demonstrated that, although not statistically similar to Sumai 3, levels of disease were below 10% in some of the Chinese genotypes. Follow up experiments using reporter strains of *Fusarium graminearum* explored the accumulation of fungal biomass and the expression of the gene *Tri5*, which is essential for DON biosynthesis. Fungal biomass levels were not significantly different between genotypes; however expression of the *Tri5* gene was significantly lower in the genotype Alsen.

A previously developed wheat leaf seedling bioassay was also explored. Scanning electron microscopy revealed the presence of fungal hyphae in advance of the visible lesion during the infection course of *F. culmorum*. Inoculation with a *Tri5* mutant strain of *F. graminearum* demonstrated that a lack of mycotoxin production altered the lesion type. This project has successfully identified potential novel resistance mechanisms and the future prospects for the control of this disease are discussed.

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CHAPTER 1: INTRODUCTION

1.1 Fusarium Ear Blight

1.1.1 Epidemiology

Wheat (*Triticum* species) is one of the most important cereal crops worldwide and together with rice and maize provides 60% of our calorie intake (Gill et al., 2004). In 2004, production totalled 2 billion tonnes worldwide (Gill et al., 2004). Wheat production is predominantly conducted in China, India, USA, Central Europe, Russia, Australia, South America and South Africa (Oerke, 2006; Varshney et al., 2006). Pests and pathogens are responsible for losses in wheat production of nearly 50% and more specifically, fungal and viral diseases can reduce yields by 18% (Oerke, 2006). One of the most important diseases of wheat worldwide is Fusarium Ear Blight. Fusarium Ear Blight (FEB), also known as Fusarium Head Blight, is a disease of small grain cereals caused by *Fusarium* spp. There are up to 17 known causal species of FEB which often occur as a complex with the predominant species worldwide being *F. graminearum* (teleomorph *Gibberella zeae*), *F. culmorum* and *F. avenaceum* (teleomorph *Gibberella avenacea*) (Stack and McMullen, 1985; Parry et al., 1995; Bottalico and Perrone, 2002; Fernandez and Chen, 2005) . This disease has re-emerged globally and is now a devastating disease of wheat, barley, maize and other small grain cereals (McMullen et al., 1997), causing serious yield losses once every three to five years (Champeil et al., 2004). Problems associated with FEB are two fold. Firstly, shrivelling of infected grain leads to substantial yield losses, up to 70% in epidemic years (Martin and Johnston, 1982). Secondly, contamination of grain with fungal mycotoxins results in a reduction in end-use quality and is also a concern for both food and feed safety (Wei et al., 2005). Fusarium damaged grain are often associated with beer gushing (Schwarz et al., 1996; Zapf et al., 2006) and the destruction of starch granules and storage proteins leading to reduced loaf volume and poor bread making (Bechtel et al., 1985; Nightingale et al., 1999; Jackowiak et al., 2005; Wang et al., 2005; Yu et al., 2008d).

F. graminearum is the most commonly isolated species (Markell and Francl, 2003; Klix et al., 2008) and predominates in warmer regions, such as the USA, Canada, Australia, and Central Europe, whereas *F. culmorum* is found in cooler maritime regions such as North Western Europe (Parry et al., 1995; Doohan et al., 1998; Bottalico and Perrone, 2002; Moss, 2002). However, yearly changes in *Fusarium* species profile in specific countries/regions may be influenced by several factors such as host species susceptibility, meteorological conditions, fungicide treatments, and agricultural practices (Parry et al., 1995; Simpson et al., 2001; Bottalico and Perrone, 2002; Xu et al., 2005; Klix et al., 2008).

In the UK, the main species responsible for FEB are *F. culmorum*, *F. poae*, *F. avenaceum* and the non mycotoxin producing species *Microdochium nivale* var. *majus*, (recently reclassified as *Microdochium majus* (Glynn, 2005)) although *F. graminearum* has started to increase to significant levels in the last five years (www.cropmonitor.co.uk, Waalwijk et al., 2004; Xu et al., 2005; Gosman et al., 2007). During the 2006/2007 season, there was an unusually high incidence of the disease, most likely caused by wet weather at anthesis (www.cropmonitor.co.uk). The disease had lowest incidence in the Yorkshire and Humberside region and the highest in north east and north west of England. The majority of isolations of *F. culmorum* and *F. graminearum*, the two species considered to be most important in mycotoxin contamination, were found south of a line stretching from Lincolnshire to Gloucestershire (www.cropmonitor.co.uk). On a worldwide scale, the incidence of FEB is increasing due to minimal tillage, which provides the pathogen with plant residues on which to over-winter (Windels, 2000). An increase in the percentage of land used for growing small grains and limited host resistance in adapted cultivars have also contributed (Windels, 2000).

FEB is a monocyclic disease (Fernando et al., 1997) and infection occurs when inoculum is present during periods of high humidity. Epidemic severity depends on the quantity of inoculum present during this period (Sutton, 1982). *Fusarium* spp. over-winter on infected crop debris from the previous season on the soil surface (Khonga and Sutton, 1988; Champeil et al., 2004; Bateman, 2005). Survival is either through saprophytic growth (Burgess, 1981; Goswami and Kistler, 2004) or the formation of thick-walled

chlamydospores or conidia (Nyvall, 1970; Burgess, 1981). Survival is generally better on plant material which resists decomposition and is on the soil surface, with lower temperatures favouring survival (Sutton, 1982; Inch and Gilbert, 2003).

1.1.2 Infection Process

FEB develops under favourable warm and humid conditions which are ideally 25ºC and 100% relative humidity for 24 hours post spore deposition (Sutton, 1982; Parry et al., 1995). Spores (conidia and ascospores) dispersed by rain-splash or wind (Jenkinson and Parry, 1994; Parry et al., 1995) are deposited on the inside of spikelets when this structure opens during anthesis. Wheat plants are most susceptible during anthesis, but infection can occur up to the soft dough stage (Schroeder and Christensen, 1963; McKeehen et al., 1999; Windels, 2000). Spores germinate on wheat surfaces within 6-12 hours (Wanjiru et al., 2002). Anthers have been noted as the most susceptible part of the flower. Choline and glycine betaine, which are found at particularly high concentrations in the anther tissue, stimulate hyphal branching which is thought to aid infection (Strange and Smith, 1971; Strange et al., 1974). A network of thin primary hyphae is formed 24 hours post inoculation on the inner surfaces of the lemma, glumes and palea, as well as on the ovary surface, particularly in the presence of pollen (Kang and Buchenauer, 2000b; Pritsch et al., 2000; Wanjiru et al., 2002). The inner surfaces display more hyphal growth than the outer surfaces and this maybe due to the presence of a waxy layer on the outer surface (Kang and Buchenauer, 2000c). Penetration without appressorium formation occurs within 24-36 hours on the inner surface of the lemma and on the ovary (Kang and Buchenauer, 2000b). The fungus penetrates the cuticle of the lemma epidermis and then grows inside the cell walls or penetrates the epidermal cells (Kang and Buchenauer, 2000b; Wanjiru et al., 2002). The hyphae spread intercellularly in the lemma and inter- and intracellularly throughout the pericarp of the ovary (Kang and Buchenauer, 2000b; Wanjiru et al., 2002). Five days post infection (dpi), the fungus spreads through to other spikelets in the ear, either externally during damp conditions (Goswami and Kistler, 2004), or more commonly internally via the rachis at the base of the infected spikelet (Kang and Buchenauer,

2000b; Wanjiru et al., 2002). Spread can be in either direction through the vascular bundles and cortical tissues leading to premature death of the ear (Schroeder and Christensen, 1963; Kang and Buchenauer, 2000b; Wanjiru et al., 2002). Conidiophores are produced 5 dpi on the inner surfaces of the lemma, glume and palea and then later on the outer surfaces (Kang and Buchenauer, 2000c). Coupled with this growth is extensive cell damage. Enzyme-gold and immuno-gold labelling techniques have demonstrated that important cell wall components such as cellulose, xylan and pectin are altered, probably by the use of cell wall degrading enzymes such as cellulases, xylanases and pectinases (Kang and Buchenauer, 2000b). This damage was also seen in advance of the hyphae, suggesting that secreted enzymes are enhancing hyphal growth (Kang and Buchenauer, 2000b). Symptoms initially appear as premature bleached ears, and further damage is seen with the shrivelled mycotoxin contaminated grain at harvest (Figure 1.1)

1.1.3 Trichothecene biosynthesis

Some pathogenic fungi are characterised by the ability to produce a suite of secondary metabolites during the infection process. The most economically important metabolites include toxins produced by plant pathogens, such as the aflatoxins and ochratoxin synthesised by *Aspergillus* spp., and ergot alkaloids produced by *Claviceps purpurea* (Bennett and Klich, 2003). During infection, *Fusarium* spp. produce various mycotoxins. The sesquiterpenoid trichothecenes deoxynivalenol (DON) and nivalenol (NIV), produced by *Fusarium graminearum* and *F. culmorum*, and T-2 toxin, produced by *F. sporotrichioides*, have received the most attention due to their potent toxicity. By understanding the biochemical and genetic control of the biosynthetic pathway in the pathogen it may be possible to develop suitable fungicides or resistant plants (Alexander et al., 1997). Trichothecene biosynthesis begins with the cyclisation of *trans, trans*-farnesyl pyrophosphate, catalysed by trichodiene synthase to form trichodiene (Desjardins et al., 1993). A series of oxygenations, isomerisations, cyclisations and esterifications follows as shown in Figure 1.2. (Desjardins et al., 1993). Elucidation of the genetic control of trichothecene biosynthesis began using *F. sporotrichioides* (Hohn and Beremand, 1989) and later *F.*

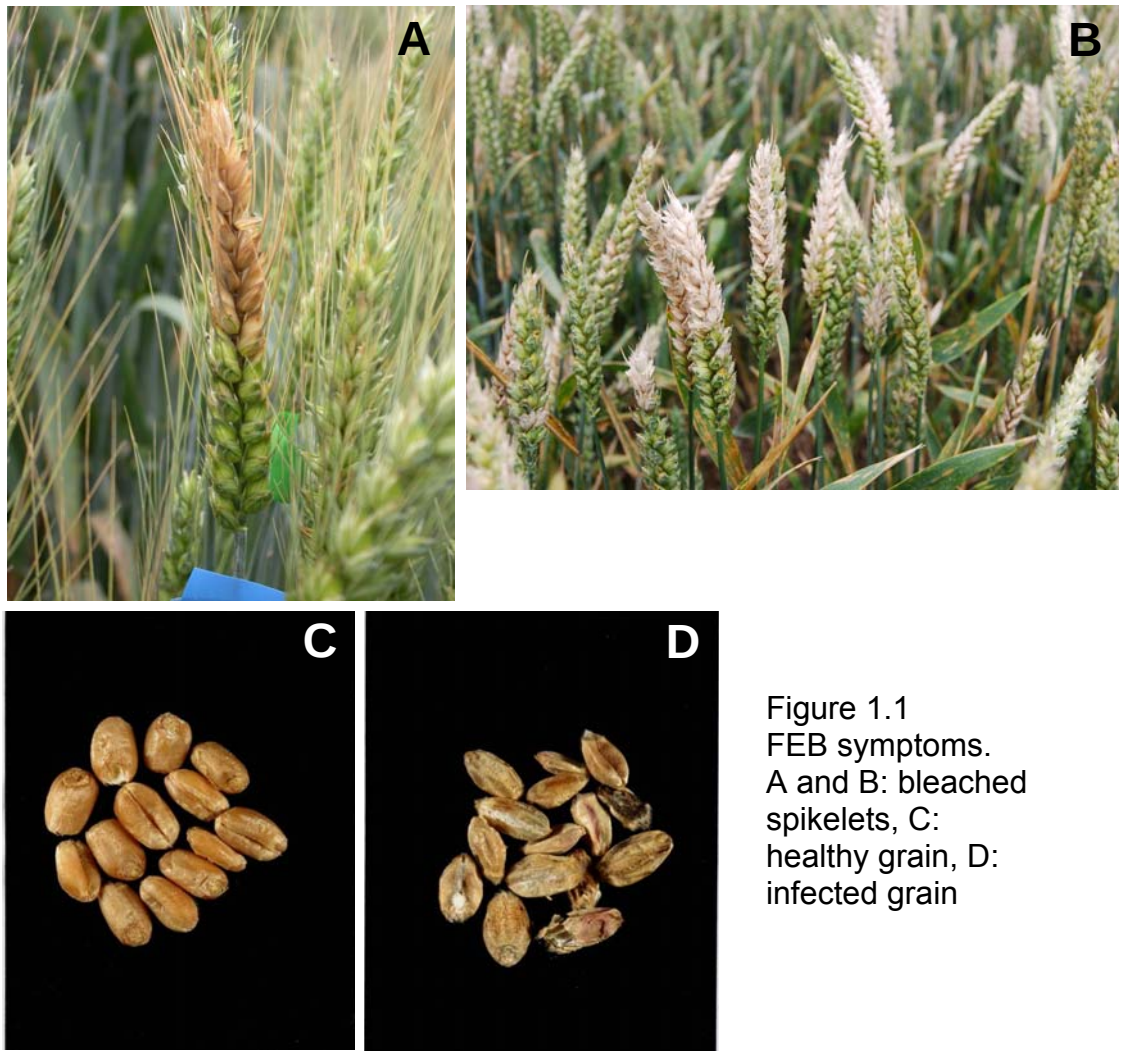


Figure 1.1
FEB symptoms.
A and B: bleached spikelets, C: healthy grain, D: infected grain

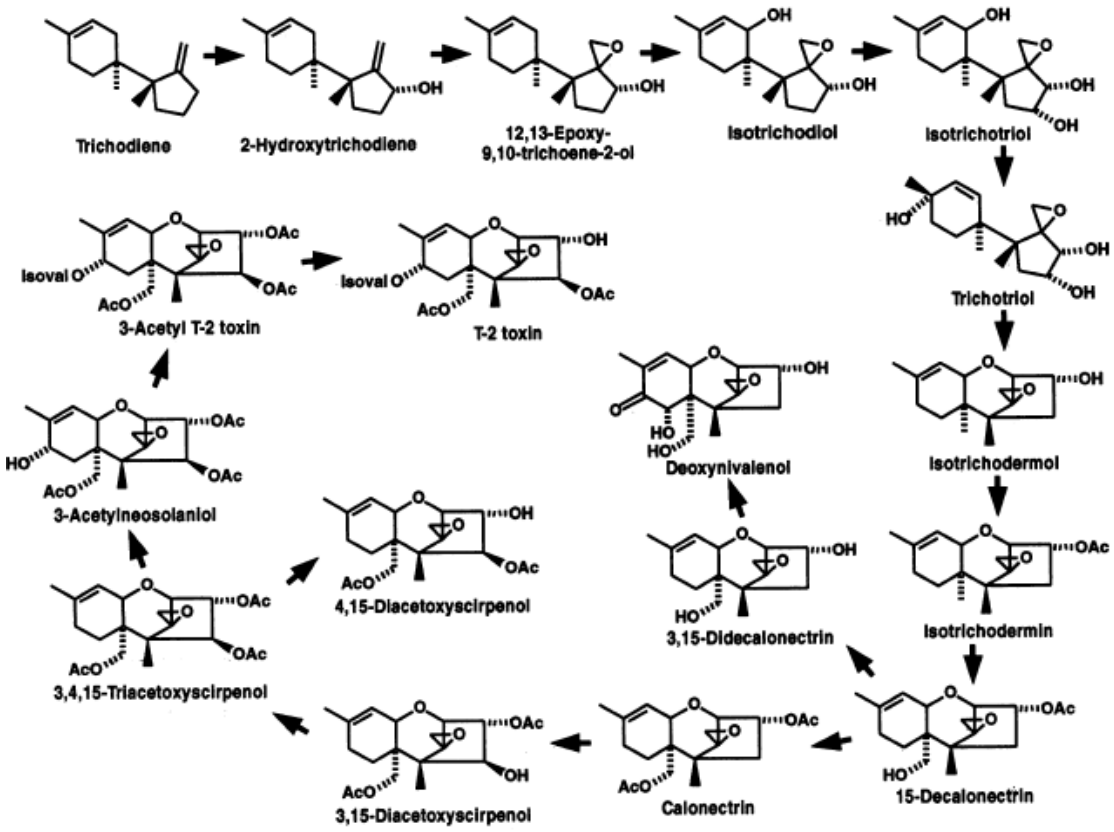


Figure 1.2 The trichothecene biosynthetic pathway (Desjardins et al, 1993)

graminearum. Genes involved in the biosynthetic pathway are summarised in Table 1.1. There are currently thought to be four main gene clusters involved in biosynthesis:

- The core 12-gene cluster containing *Tri8-Tri14* (Kimura et al., 2003a; Kimura et al., 2003b; Brown et al., 2004) (Figure 1.3)
- A two gene cluster *Tri16* and *Tri1* (Brown et al., 2003; Meek et al., 2003; Peplow et al., 2003b)
- A single gene locus *Tri101* (Kimura et al., 1998c; Kimura et al., 1998b; Brown et al., 2001; Alexander et al., 2004)
- A single gene locus containing *Tri15* (Zhou et al., 2003; Alexander et al., 2004)

The location of action of some of the gene products in the biosynthetic pathway are highlighted in Figure 1.4. The *Tri* genes are under the regulation of *Tri10* with co-regulation of *Tri6* downstream (Proctor et al., 1995b; Hohn et al., 1999; Peplow et al., 2003a). The exceptions to this are *Tri15* (Alexander et al., 2004) and *Tri101* (Kimura et al., 1998a).

Trichothecenes are a broad group of chemicals with a central nucleus of the tricyclic trichothecene molecule (Desjardins et al., 1993). They are divided into four groups (A-D) depending on the chemical properties and the producing organism. *Fusarium* spp. produce type A and type B trichothecenes which are differentiated by the presence of an oxygen or carbonyl functional group at C-8 respectively. Type A trichothecenes include T-2 toxin and Type B include DON and NIV and the acetylated derivatives 3-acetyldeoxynivaneol and 15-acetyldeoxynivalenol (Ueno et al 1973 and Ueno et al 1985 in Rocha et al., 2005). DON, NIV, and T-2 toxin differ structurally, as shown in figure.1.5 (Adapted from Brown *et al* (2002)). DON production is achieved through the inactivation of *Tri16* in *F. graminearum*, which gives a C-8 keto group instead of a C-8 ester, producing DON instead of T-2 toxin (Brown et al., 2003). Differences in mycotoxin production within a species also form the basis of classification of isolates according to chemotypes. Chemotypes are classifications of the fungi based on the predominant mycotoxin produced, and can be one of NIV, 3-ADON and 15-ADON chemotypes (Figure 1.4) (Ichinoe et al., 1983; Miller et al., 1991).

Table 1.1 Genes involved in the biosynthesis of trichothecene mycotoxins

Gene	Gene Product	Function	References
Tri1	Cytochrome P450 monooxygenase	C-7 and C-8 oxygenation	(Brown et al., 2003; Meek et al., 2003; McCormick et al., 2004; McCormick et al., 2006a)
Tri3	15-*O*-Acetyltransferase	Conversion of 15-decalonectrin to calonectrin	(McCormick et al., 1996; Kimura et al., 2003b)
Tri4	Cytochrome P450 monooxygenase	Multifunctional monooxygenase, catalyses four steps in the conversion of trichodiene to isotrichodermin and trichothecene	(Hohn et al., 1995; McCormick et al., 2006b; Tokai et al., 2007)
Tri5	Trichodiene synthase	Catalyses isomerization-cyclisation of farnesyl pyrophosphate to form trichodiene	(Hohn and Vanmiddlesworth, 1986; Hohn and Beremand, 1989; Proctor et al., 1995a; Proctor et al., 2002)
Tri6	Cys_2-His_2 zinc finger protein: transcription factor	Positive regulator trichothecene pathway genes, binds to promoter regions of trichothecene pathway genes inducing expression	(Proctor et al., 1995b; Hohn et al., 1999)
Tri7	3-acetyltrichothecene 4-*O*- (C-4)acetyltransferase	Acetylation of the oxygen on C-4 of T-2 toxin, responsible for further modification of NIV	(Brown et al., 2001; Lee et al., 2001; Lee et al., 2002)
Tri8	Trichothecene C-3 deacetylase	Deacetylation at C-3, removes the C-3 acetyl group, which increases toxicity	(Brown et al., 2001; McCormick and Alexander, 2002; Kimura et al., 2003b)
Tri9	No similarity to previously described proteins		(Brown et al., 2001)
Tri10	Regulatory gene - unknown protein	Positive regulator trichothecene pathway genes, regulates transcription of *Tri6*	(Brown et al., 2001; Tag et al., 2001; Peplow et al., 2003a)
Tri11	Cytochrome P450 monooxygenase	Hydroxylation of C-15	(McCormick and Hohn, 1997; Alexander et al., 1998)

Table 1.1 continued

Gene	Gene Product	Function	References
Tri12	Trichothecene efflux pump	Transporting trichothecenes out of the cell	(Alexander et al., 1999)
Tri13	Cytochrome P450 oxygenase	Addition of C-4 oxygen	(Lee et al., 2001; Brown et al., 2002; Lee et al., 2002)
Tri14	No similarity to previously described proteins	Not required for trichothecene biosynthesis	(Brown et al., 2002; Kimura et al., 2003b; Dyer et al., 2005)
Tri15	Cys_2-His_2 zinc finger protein: transcription factor	Not necessary for T-2 synthesis. Possible negative regulator of some biosynthetic genes	(Peplow et al., 2003a; Alexander et al., 2004)
Tri16	Acyltransferase	Esterification of the C-8 hydroxyl group	(Brown et al., 2003; Meek et al., 2003; Peplow et al., 2003b)
Tri101	Trichothecene 3-*O* acetyltransferase	Confers resistance to trichothecenes through 3-*O*-acetylation of the trichothecene ring, plays a role in self protection but is not essential for self-defence. Also converts isotrichodermol to isotrichodermin and is required for the biosynthesis of T-2 toxin.	(Kimura et al., 1998c; Kimura et al., 1998a; McCormick et al., 1999)
Tri102	Trichothecene efflux pump	Homologue of the *F. sporotrichioides Tri12* in *G. zeae*	(Wuchiyama et al., 2000)

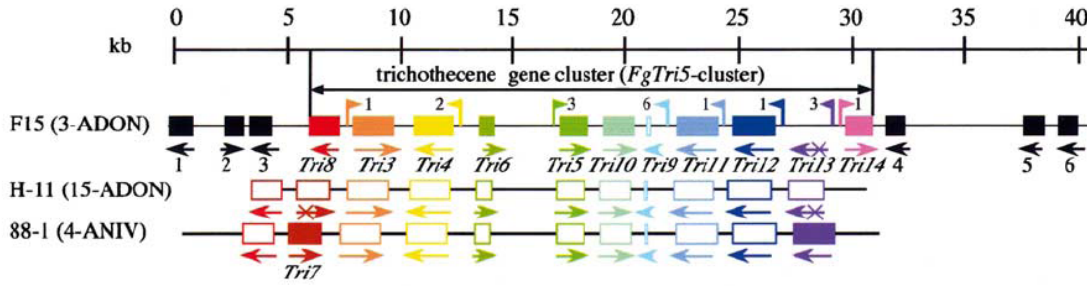


Figure 1.3 The core trichothecene biosynthesis gene cluster. Comparison is made between species producing different mycotoxins (different chemotypes). Genes of the same colour are the same biosynthetic gene. *Fusarium* strain numbers are given on the left (F15, H-11, and 88-1), with chemotypes given in parentheses. The different mycotoxins are produced through the selective expression of the *Tri7* and *Tri13* genes. Arrows = direction of transcription, X = inactivation. Flags indicate *Tri6* transcription factor binding sites (Kimura et al, 2003).

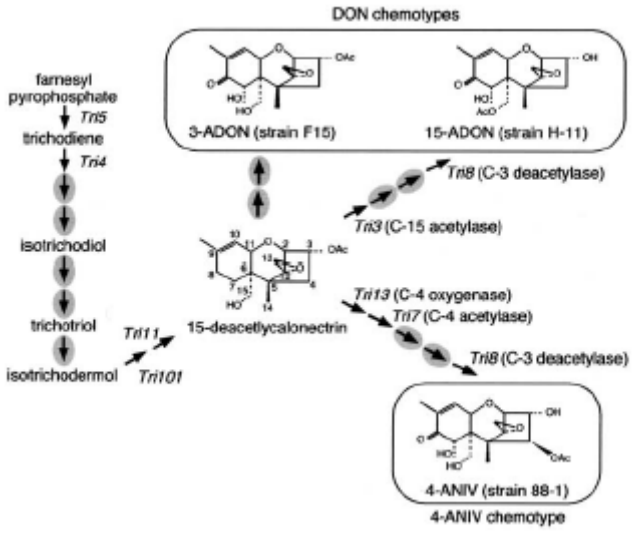
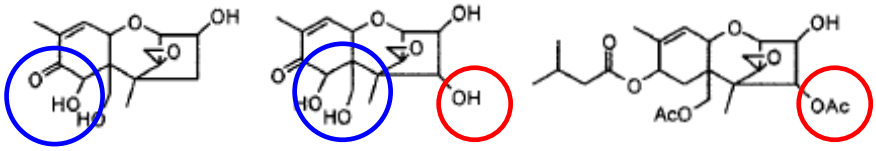


Figure 1.4 The biosynthetic pathway of DON mycotoxin showing the location of operation of some of the *Tri* gene products. *Fusarium* strain numbers are given in parentheses and demonstrate the different pathways involved in producing different chemotypes. Grey ovals indicate that the gene for that step is currently unknown (from Kimura et al 2003)



DON NIV T-2 Toxin

Figure 1.5 Chemical structures of DON, NIV and T-2 toxin. Red circles highlight the oxygen moiety present on C-4 in NIV and T-2 toxin. Blue circles highlight the additional hydroxyl group at C-7 and hydroxyl group instead of isovalerate group on C-8 on DON and NIV compared to T-2 toxin (Adapted from Brown *et al* (2002)).

Different chemotypes are achieved through the combination of *Tri* genes expressed (Figure 1.3). *Tri7* determines the production of NIV or DON: the absence of *Tri7* leads to the production of DON instead of NIV. Following on from this, the action of the Tri3 and *Tri8* gene products lead to the production of 15-ADON instead of 3-ADON (Figure 1.4 and Lee et al., 2002).

The chemotype distribution of *F. culmorum* isolates in the UK was investigated and it was found that the NIV chemotype predominated in the south and west of England and Wales, whereas the DON chemotype predominated in the north and east of England (Jennings et al., 2004). No regional differentiation was seen for the chemotype produced by *F. graminearum*. Chemotype distribution in a large-scale experiment in China demonstrated differences according to geographical location, with NIV chemotypes predominantly found in the high altitude mountainous regions, and DON chemotypes found in the middle and lower regions (Yang et al., 2008).

1.1.4 Mycotoxin mode of action and accumulation *in planta*

DON is considered a health concern. Contaminated grain can cause feed refusal and vomiting in animals (Moss, 2002). Trichothecenes can cause the inhibition of protein synthesis, through the inhibition of the peptidyl transferase active site within the ribosome (Feinberg and McLaughlin, 1989; Rotter et al., 1996) and is thought to induce apoptosis (Maresca et al., 2002). Toxicity is conferred by the epoxide at C-12 and C-13 of the DON molecule (Desjardins et al., 1993; Rotter et al., 1996); de-epoxidation can result in non or less toxic metabolites (Zhou et al., 2008). DON is phytotoxic, causing inhibition of coleoptile growth (Wang and Miller, 1988).

DON production *in planta* is detected 36 hours post inoculation. Cells in close contact with fungal hyphae on the lemma and ovary showed toxins to be present. Toxins have been found in the cytoplasm, chloroplasts, plasmalemma, cell wall, and vacuoles, and sometimes associated with the endoplasmic reticulum and ribosomes. Toxins have also been detected in xylem and phloem tissues where there were no hyphae present (Kang and Buchenauer, 1999). Furthermore, high levels of DON have been found in the rachis tissue, in some cases ahead of spikelets showing DON accumulation.

Unfortunately, the authors did not detail the progress of visible symptoms, which would allow for the determination of mycotoxin contamination in comparison to the visible symptoms. Interestingly, DON levels were much lower in the regions above the point of inoculation. Previous work has suggested that the fungus blocks the vascular tissue (Lemmens et al., 1997), preventing water and nutrients reaching the upper portion of the ear. As a water soluble metabolite (Snijders, 1994), it is possible that DON is also prevented reaching the upper parts, thereby minimising the accumulated levels detected (Savard et al., 2000).

DON accumulates to concentrations up to 600 ppm in grain originating from natural infections (Sinha and Savard, 1997). Immunocytochemical localisation has shown that DON is water soluble and can be translocated in the xylem and phloem (Kang and Buchenauer, 1999), which can lead to physiological effects in other parts of the plant, with DON found in other floral tissues not previously invaded by the fungus (Snijders and Krechting, 1992; Kang and Buchenauer, 1999; Snijders, 2004). To investigate the role that DON plays in infection, a *F. graminearum* mutant which is unable to produce DON through disruption of the *Tri5* gene was developed. The strain displayed normal growth and development *in vitro*. Inoculation of wheat ears with this strain resulted in reduced virulence however, with bleaching seen in inoculated spikelets, but no further spread (Proctor et al., 1995a; Bai et al., 2001a; Cuzick et al., 2008a). Interestingly, when barley was inoculated with a DON non-producing strain, no reduction in virulence was evident, demonstrating that the effect of DON is dependent on the host species (Maier et al., 2006). When inoculated with a *Tri5* mutant, cell-wall thickenings were evident in wheat tissues at the base of the inoculated spikelet. These thickenings were not seen in wild-type *F. graminearum* inoculated spikelets, suggesting that DON functions in suppressing this host resistance response (Jansen et al., 2005). Together, this evidence suggests that trichothecenes, whilst not strictly essential for fungal pathogenicity, can act as virulence factors during disease spread (Proctor et al., 1995a; Mirocha et al., 1997).

The production of DON is influenced by the environment. A positive correlation has been found between DON production and rainfall, and a negative correlation between temperature and DON production (Vanco et al.,

2007). DON formation also increased when the conditions were wet for up to at least 3 days post inoculation (Lacey et al., 1999). Three critical periods were also identified which influenced the production of DON under field conditions: four to seven days before heading, three to six days after heading, and seven to ten days after heading. In all cases, an increase in the number of days with rainfall lead to an increase in the amount of DON produced (Hooker et al., 2002). *In vitro*, optimal conditions for DON production were determined to be 0.97 water activity (a_w) at 15 ºC and 0.99 a_w at 25 ºC for *F. culmorum* and 0.99 a_w at 15 ºC and 0.98 a_w at 25 ºC for *F. graminearum* (Hope et al., 2005).

1.1.5 Control Methods

There are several methods for controlling FEB, each with potential advantages and disadvantages. No single method adequately controls FEB, and a more integrated approach should lead to better control. The use of a crop rotation which avoids the planting of maize before wheat reduces the potential debris on which the fungus can overwinter (Burgess, 1981; Teich and Hamilton, 1985; Snijders, 1994; Inch and Gilbert, 2003). Ploughing in residues also reduces the inoculum source for the following year (Blandino et al., 2006; Pereyra and Dill-Macky, 2008). These two methods are both only viable to a certain extent. Conservation tilling used in many parts of the world, including the USA, means that infested residues remain at or near the soil surface (McMullen et al., 1997).

The application of fungicides has only been met with limited success and is costly and highly dependent on correct timing of application (Jones, 2000; Hope and Magan, 2003; Shen et al., 2003b). Currently, the best treatments on the market are based on tebuconazole (Homdork et al., 2000; Mesterhazy et al., 2003; Sip et al., 2007; Pirgozliev et al., 2008), propiconazole (Martin and Johnston, 1982) or a mixture of the two (Paul et al., 2008). These fungicides are of the azole class, which target the ergosterol synthesis pathway in fungi (Cools and Fraaije, 2008). Fungicide application is further complicated by the problem that in some cases, the mycotoxin content can be seen to increase in relatively uninfected ears, however, this is most

likely due to the selective removal of some *Fusarium* spp. but not others (Simpson et al., 2001).

Biological control of FEB includes the use of the potential antagonists *Clonostachys rosea* (Luongo et al., 2005), the yeast *Cryptococcus nodaensis* (Khan et al., 2004), *Fusarium equiseti* (Dawson et al., 2004) and *Phoma beate* (Diamond and Cooke, 2003) amongst others. However, survival of the antagonist under field conditions on the ear is a limiting factor in this approach (Gilbert and Fernando, 2004).

Post-harvest control can also limit further contamination of grain. Poor grain storage conditions can cause mycotoxin levels to increase post-harvest (Aldred and Magan, 2004). Important considerations include moisture, temperature, and physiological condition of the grain at harvest, and drying regime, storage type and hygiene post-harvest (Aldred and Magan, 2004). Control methods to limit this post-harvest accumulation include the potential bulk-drying of grain and assessment of grain for presence of FEB. Grain moisture has a large effect on further spoilage and mycotoxin contamination, and lowering moisture to < 0.70 a_w will limit this contamination. However, grain is usually harvested at levels higher than this and bulk drying of grain can be problematic (Aldred and Magan, 2004).

1.2 Floral Infecting Pathogens

Plant pathogens are capable of infecting different organs of the host. Some display no specificity, such as the potato late blight pathogen *Phytophthora infestans*, which can infect leaves, stems, roots, and tubers (Birch and Whisson, 2001). By contrast, *Mycosphaerella graminicola*, the causal agent of septoria leaf blotch on wheat, is more specific and only infects the leaves (Palmer and Skinner, 2002). The eyespot fungi *Oculimacula yallundae* and *Oculimacula acuformis* similarly only infects the stem bases of cereals (Lucas et al., 2000).

Pathogens infecting the floral tissues are less common than those infecting other organs. The best known example is the ergot fungus *Claviceps purpurea*, which exclusively infects the floral tissue (Tudzynski and Scheffer, 2004). At cereal anthesis, wind-borne ascospores are blown into open spikelets and initiate infection. The ovary is targeted and colonisation

occurs, followed by contact with the rachis, from which nutrients can be derived for the formation of the sphacelium containing the conidiospores. Ultimately, infection by this fungus results in the replacement of grain with black sclerotia which are the overwintering structure for this fungus and contain toxic alkaloids (Tudzynski and Scheffer, 2004).

Like *Claviceps purpurea*, infection with the wheat loose smut pathogen *Ustilago nuda* f.sp. *tritici* also results in the replacement of grain with teliospores (Jones and Clifford, 1978). After arriving on a stigma or pericarp, the spore germinates to form a promycelium, which after fusing with another compatible promycelium, forms branching hyphae. The hyphae penetrate the grain and remain dormant until the seed is sown. After germination of the seedling, the mycelium grows up the plant, colonising the nodes progressively. Eventually, the rachis becomes replaced by a mass of spores produced by the mycelium, and on emergence, the spores are wind-blown to neighbouring ears (Jones and Clifford, 1978).

Karnal bunt, caused by *Tilletia indica*, also infects the floral tissue. Grain is partially filled with teliospores on a varying scale from minimal infection, through to complete replacement of grain tissues with teliospores (Carris et al., 2006). Life cycle stages are again similar to that of FEB, as teliospores on the soil surface germinate to form basidiospores and sporidia. Sporidia are deposited on the spikelets and subsequent hyphae penetrate stomata. Similar to ergot infection, the ovary is targeted, however, in Karnal bunt, the embryo remains alive, with teliospore formation taking place in outer tissue layers such as the pericarp. Only in severe infections is the embryo killed, and in most cases infected grain are still able to germinate (Carris et al., 2006).

Stagonospora nodorum is less organ-selective and is capable of infecting both ears and leaves of wheat and barley (Solomon et al., 2006). Leaf infection takes place through the penetration of the cuticle and stomata, resulting in the formation of chlorotic lesions and the appearance of pale brown pycnidia. Ear infection, known as glume blotch, occurs when rain-splashed pycnidiospores are deposited on the ear (Solomon et al., 2006), therefore contrasting with *Fusarium* infection which only saprophytically colonise the leaves without causing infection.

Fusarium spp. are capable of infecting a wide range of host plants and organs. In addition to infecting the ear, *F. graminearum* can infect the stem bases of cereal crops, causing brown foot rot, and can infect seedlings to cause seedling blight (Parry et al., 1995). Although macroconidia of *F. graminearum* and *F. culmorum* are often found on leaves of cereal plants, leaf infection by this fungus is never observed (R. Gutteridge, Rothamsted Research, pers. comm.). In Australia, FEB rarely occurs due to the drier growing conditions. However *F. graminearum* along with *F. pseudograminearum* are capable of causing crown rot in wheat causing lesions on the base of stems and reducing yields by up to 100% (Stephens et al., 2008). The related species *Fusarium oxysporum* infects a wide range of hosts and primarily causes disease through the infection of roots (Rep et al., 2002).

1.3 Main Features of Plant Defence against Invading Pathogens

In the majority of cases, plants are able to resist invading pathogens. Plants defend themselves through a layered defence system consisting of preformed physical and chemical barriers and active defences. Entry to the host can be through stomata, wounds or by direct penetration, and at this point the first line of defence is the plant cell wall. The cell wall is reinforced with suberin and lignin, and the presence of preformed inhibitors such as phenols, alkaloids, glycosides, and saponins are, in most cases, sufficient to keep invading micro-organisms from causing a visible infection (Lucas, 1998). The relationship between the host and pathogen continually evolves, and has been likened to an arms race. In response to this physical and chemical barrier, pathogens have developed a battery of cell wall degrading enzymes. Penetration attempts by pathogens in an incompatible reaction are sometimes halted by the host by the deposition of papillae at the attempted entry point. Reinforcements of the cell walls also occur through the deposition of callose and suberin, lignification, and oxidative cross-linking (Lucas, 1998). Compatible reactions between host and pathogen result in the cell wall being breached and the plasma membrane being exposed. Surface receptors on the plasma membrane function in the detection of pathogen-associated

molecular patterns (PAMPs). PAMPs can include cell-surface components of Gram-negative bacteria such as lipopolysaccharide and flagellin, and the fungal components chitin and ergosterol (Nurnberger et al., 2004; Zipfel and Felix, 2005). Recognition at this point results in the activation of PAMP-triggered immunity (PTI), which will halt some invading microorganisms through the activation of pathogenesis-related (PR) proteins, deposition of callose and the production of reactive oxygen species (Nurnberger et al., 2004; Zipfel and Felix, 2005). However, some microorganisms are able to suppress PTI either through interference with plasma membrane recognition or through secretion of effector proteins into the cytosol (Chisholm et al., 2006). Effectors can be deployed through the use of the type III secretion system in bacteria, or through the haustoria in fungi. Effectors can also interfere with the hypersensitive response and hormone signalling such as the salicylic acid, ethylene, and jasmonic acid pathways. Recognition of these effectors by the resistance (R) proteins or gene products in plants results in effector triggered immunity (ETI) and is the basis for gene-for-gene resistance (Jones and Dangl, 2006). The gene-for-gene hypothesis suggests that there are cognate plant *R* genes to avirulence (effector) genes in the pathogen (Flor, 1971). Interaction between the correct combination results in the mounting of a resistance response and infection is halted. In the absence of the cognate *R* gene, the interaction continues in favour of the pathogen and infection develops. There are two main classes of *R* gene (Hammond-Kosack and Parker, 2003). Nucleotide-binding leucine rich repeat (NB-LRR) are the largest class and can be further divided according to the N terminal domain: coiled coil (CC) or toll-interleukin-1 (TIL). A second class of *R* gene, extracellular leucine rich repeat (eLRR) *R* genes code for proteins that include receptor like proteins (RLP) and receptor like kinases (RLK) (Hammond-Kosack and Kanyuka, 2007). Binding between the effector and R protein is either direct or indirect. Direct interaction is seen in tomato with the Pto protein kinase, which interacts with the bacterial effectors AvrPto and AvrPtoB (Hammond-Kosack and Kanyuka, 2007), however, these interactions are rare. Indirect interaction forms the basis of the guard hypothesis (Van der Biezen and Jones, 1998). This suggests that the R proteins act more as a guard of the target of the Avr protein. In the presence of the R protein, any disturbance

of the target is detected and the defences are activated. If the R protein is absent or inactivated, the Avr protein can interact with the target undetected and colonisation ensues, which suggests that the R protein functions more like an antennae monitoring for disturbances (McDowell and Simon, 2006). More recently, the "Decoy" model for *R/Avr* gene interactions has been developed. This suggests that some of the targets of the pathogen effectors could act as decoys. It is the decoys themselves that are associated with the R proteins, triggering the defence response as necessary. This subtle difference suggests that the R protein is not associated with the target *per se*, but is associated with a target with a similar conformation. This decoy target could arise through the gene duplication events or the mimicry of targets (van der Hoorn and Kamoun, 2008). Further experimental evidence is necessary to explore this model. *R*-gene-mediated resistance is associated with the oxidative burst, hypersensitive response and the activation of plant hormone pathways involving salicylic acid, jasmonic acid and ethylene. In general, salicylic acid is active against biotrophs, and ethylene and jasmonic acid work together against necrotrophs. There is a good degree of overlap however, and these pathways should be seen more as a network with significant cross-talk (Glazebrook, 2005).

1.4 Natural Resistance to FEB

Cereal species resistance to FEB contrasts to that observed in many other pathosystems which display a more conventional gene-for-gene resistance (McDonald and Linde, 2002), where only specific races of the pathogen are pathogenic on a limited range of host genotypes. Resistance to FEB is not only race-non-specific, but also species-non-specific (Snijders and Van Eeuwijk, 1991; Van Eeuwijk et al., 1995; Bai et al., 1999; Mesterhazy et al., 2005) and it is well documented that wheat cultivars differ only by varying degrees in their resistance to *Fusarium* Ear Blight (reviewed by Parry et al., 1984; Bai and Shaner, 2004). Morphological features associated with resistance to FEB include: (feature in brackets denotes resistant character) height (tall), presence or absence of awns (absence), spikelet density (low), peduncle length (long), flower opening (narrow), flowering time (short) (Mesterhazy, 1995; Parry et al., 1995; Rudd et al., 2001; Somers et al., 2003;

Snijders, 2004; Gilsinger et al., 2005). These features each reduce fungal spread and invasion by escape mechanisms, for example a lower spikelet density will impede the spread of the fungus in comparison to an ear with more tightly packed spikelets. Physiological resistance within the ear has also been described and can be divided into five types:

Type I: Resistance to initial infection (Schroeder and Christensen, 1963)

Type II: Resistance to disease spread (Schroeder and Christensen, 1963)

Type III: Degradation of or resistance to toxin accumulation (Miller et al., 1985; Miller and Arnison, 1986; Wang and Miller, 1988; Snijders and Perkowski, 1990; Mesterhazy, 1995)

Type IV: Resistance to kernel infection (Mesterhazy, 1995; Mesterhazy et al., 1999)

Type V: Yield tolerance through tolerance of DON mycotoxin (Wang and Miller, 1988; Mesterhazy, 1995; Mesterhazy et al., 1999)

Type I resistance is highly influenced by environmental conditions and less repeatable due to a high amount of non-genetic variation and is therefore not well characterised (Bai et al., 1999; Yu et al., 2008d). Type II resistance, because it is more stable and less prone to environmental effects, has received the most attention.

Complete resistance, i.e. immunity has not been identified among the Graminae (Snijders, 1990; Snijders and Krechting, 1992; Ban and Watanabe, 2001). However, some highly resistant genotypes have been identified (Bai and Shaner, 1994). Resistant varieties are characterised by their slower and later development of symptoms and the best known example of this is the Chinese Spring variety Sumai 3 (Snijders, 2004). Sumai 3 is currently the most widely deployed source of resistance worldwide (McCartney et al., 2004). Originating from the cross between two moderately susceptible cultivars, Taiwanmai and Funo, Sumai 3 displays high levels of type II resistance and has been used extensively in breeding programmes (Bai and Shaner, 1994, 1996; Stack et al., 1997; Bai and Shaner, 2004). Other sources of resistance have been identified worldwide and include the Asian

spring wheats Nobeokabozu, Wangshuibai, Wuhan-1, the Brazilian spring wheat Frontana, and the European winter wheats Praa8, Novokrumka and Arina (Bai and Shaner, 1996; Ban and Suenaga, 2000; Gilbert and Tekauz, 2000; Lin et al., 2004; Paillard et al., 2004; Snijders, 2004; Jiang et al., 2006c; Hamzehzarghani et al., 2008b; Hamzehzarghani et al., 2008a).

Wheat genotypes not only differ in visible disease symptoms, but also their responses to DON contamination. Natural declines in the amount of DON in harvested grain in comparison to earlier in the season have been observed (Scott et al., 1984; Miller and Young, 1985) and resistant cultivars such as Sumai 3 and Frontana are well documented to resist the accumulation of DON (Wang and Miller, 1988; Miller and Ewen, 1997). Suggested resistance mechanisms have included biochemical degradation (Miller and Arnison, 1986; Wang and Miller, 1988) or resistance to protein inhibition via a modified peptidyl transferase (Miller and Ewen, 1997). Recently, the *Fhb1* resistance gene has been linked to resistance of DON through the synthesis of DON-3-*O*-glucoside (Lemmens et al., 2005).

Breeding for resistance is considered one of the best long term control measures for FEB disease (Wang and Miller, 1988). Molecular genetic studies have since aimed to map with increasing precision the loci responsible for FEB resistance. Early monosomic analyses aimed to simply identify which chromosomes were involved in resistance (Yu, 1982; Buerstmayr et al., 1999a; Buerstmayr et al., 1999b; Grausgruber et al., 1999; Zhou et al., 2002a). The next step with more advanced molecular techniques aimed to identify specific genomic regions. Quantitative traits are controlled by many different genes, and loci in the genome which contribute to the phenotype, in this case resistance, are called quantitative trait loci (QTL) (Collard et al., 2005). Different QTL contribute to the phenotype in different amounts and are typically expressed as percentage values. Coupled with the fact that the environment has a large effect on the phenotype displayed, mapping the QTL involved in FEB resistance is very difficult. QTL analysis allows this complex resistance to be broken down into smaller parts using molecular markers (Yang et al., 2005a). Molecular or genetic markers are polymorphisms in the genome between individuals and most to date have been found in non-coding regions (Collard et al., 2005). There are several types of markers which are

commonly used in QTL mapping. These are summarised below in the table 1.2 and table 1.3 summarises the major QTL identified to date.

One of the most frequently detected QTL is the one on 3BS. This can explain up to 60% of the resistance phenotype (see Table 1.3). It has been shown to be stable over different backgrounds (Zhou et al., 2003) and has been tentatively assigned a function. As well as resistance to fungal spread (type II resistance), 3BS is also associated with resistance to accumulation of toxin. DON is converted to DON-3-*O*-glucoside. This suggests that a gene within the vicinity of QTL *Qfhs.ndsu-3BS* encodes either a glucosyl transferase or it regulates the expression of a similar enzyme (Lemmens et al., 2005). Two further QTL are considered to be important in the resistance in Sumai 3, located on chromosomes 5A and 6BS (Table 1.3). More recently the QTLs on chromosomes 3BS and 6BS have been fine mapped and renamed *Fhb1* and *Fhb2*, respectively (Cuthbert et al., 2006; Cuthbert et al., 2007). *Fhb1* has also been validated in a different mapping population using near-isogenic lines (Pumphrey et al., 2007). Further, *Fhb1* has been shown to be located on the deletion bin 3BS 0.78 – 0.87 (Liu and Anderson, 2003a). A QTL on chromosome 4D coinciding with plant height has also recently received renewed attention. Initial studies prior to the discovery of the QTL suggested that the presence of the dwarfing gene allele *Rht-B1b* reduced straw height and increased disease severity (Hilton et al., 1999). Further mapping populations segregating at this locus and the homologous *Rht-D1b* locus have been tested. QTL found for resistance in the Arina x Riband population coincided with the *Rht-D1* locus for height (Draeger et al., 2007) and in the 'Mercia' near isogenic lines, the presence of the *Rht-B1b* and *Rht-D1b* alleles increased disease rating by 35 and 52%, respectively (Miedaner and Voss, 2008). Further studies in three separate mapping populations showed that the *Rht-D1b* allele increased disease rating by 22-53% (Voss et al., 2008). In the Spark x Rialto population one of the QTL for FEB resistance coincided again with the *Rht-D1* locus, and lines carrying *Rht-D1b* were compromised in type I resistance (Srinivasachary et al., 2008). In another

Table 1.2 Commonly used molecular markers which are predominantly non-genic based

Marker Type		Advantages	Disadvantages
RAPD	Random Amplified Polymorphic DNA	Fast, cheap, small amounts of DNA required, multiple loci from a single primer possible, no prior knowledge of target sequence	Problems with reproducibility, not transferable.
RFLP	Restriction fragment length polymorphism	Robust, reliable and transferable across populations.	Low polymorphism in wheat, time consuming, expensive, large amounts of DNA required.
SSR	Simple sequence repeat	Fast, cheap, efficient, accurate, can distinguish between homozygous and heterozygous genotypes (due to their codominance), abundant, chromosome specific, therefore good for physical location.	Time consuming to produce primers, resolution often requires polyacrylamide electrophoresis.
AFLP	Amplified Fragment Length Polymorphism	Multiple loci, high polymorphism	Complicated, large amounts of DNA needed.
STS	Sequence Tagged Sites	Easy to use, high through-put	Technically challenging to convert AFLP markers into STS markers.
SNP	Single nucleotide polymorphism	Abundant throughout genome, high throughput.	Not as amenable to electrophoresis detection

Adapted from (Roder et al., 1998; Hartl and Jones, 1999; Zhou et al., 2002a; Zhou et al., 2002b; Guo et al., 2003; Sun et al., 2003; Zhou et al., 2003; Feuillet and Keller, 2004; Collard et al., 2005).

Table 1.3 Major QTL identified to date for resistance to FEB in hexaploid wheat

Chromosome	R2(%)d	Resistance Type	Mapping Population	Source of Resistance	Reference
1AL	27.9	1 and 2	Arina / NK93604	NK93604	(Semagn et al., 2007)
1AL	27.9	3	Arina / NK93604	NK93604	(Semagn et al., 2007)
1B	18.7	2	Fundulea 201R (F201R)/Patterson	Fundulea 201R	(Shen et al., 2003b)
1B	12	Unknown	Dream/Lynx	Lynx	(Schmolke et al., 2005)
1B (1RS)	15.6	2	Wangshuibai / Alondra 's'	Alondra 's'	(Zhang et al., 2004)
1BL	18.1	1 and 2	Romanus / Pirat	Pirat	(Holzapfel et al., 2008)
1BL	11.9	2	Wangshuibai / Wheaton	n/s	(Zhou et al., 2004)
1BL	19.6	1 and 2	Arina / NK93604	Arina	(Semagn et al., 2007)
1BL	10.1	AUDPC	Arina / Riband	Arina	(Draeger et al., 2007)
1BS	16.5	1 and 2	Cansas/Ritmo	Cansas	(Klahr et al., 2007)
1DL	16.6	4	DH181 / AC Foremost	n/s	(Yang et al., 2005b)
2A	14	Unknown	Renan / Recital	Renan	(Gervais et al., 2003)
2A	9.0 - 11.2	4	Nanda2419 / Wangshuibai	Wangshuibai	(Li et al., 2008)
2A	11.5	2	Wangshuibai / Annong 8455	Wangshuibai	(Ma et al., 2006b)
2AL	14.3	2	Sumai-3 / Stoa	Stoa	(Waldron et al., 1999; Anderson et al., 2001)
2AS	26.7	3	Arina / NK93604	NK93604	(Semagn et al., 2007)
2B	12	Unknown	Renan / Recital	Renan	(Gervais et al., 2003)
2B	9.5 - 14.9	2	Nanda2419 / Wangshuibai	Nanda2419	(Lin et al., 2004)
2B	8.3 - 15.9	4	Nanda2419 / Wangshuibai	Nanda2419	(Li et al., 2008)
2B	7.0 - 8.6	2	Nanda2419 / Wangshuibai	Nanda2419	(Lin et al., 2004)

Table 1.3 Continued

Chromosome	R2(%)d	Resistance Type	Mapping Population	Source of Resistance	Reference
2B	11.1	AUDPC	Arina / Riband	Arina	(Draeger et al., 2007)
2BL	14.1	1 and 2	G16-92 / Hussar	G16-92	(Schmolke et al., 2008)
2BL	11	Unknown	Dream / Lynx	Dream	(Schmolke et al., 2005)
2BS	29	1	Patterson / Goldfield	Goldfield	(Gilsinger et al., 2005)
2D	10.1 - 12.3	1	Nanda2419 / Wangshuibai	Wangshuibai	(Lin et al., 2006)
2D	10.6	1 and 2	Wangshuibai / Alondra 's'	Wangshuibai	(Jia et al., 2005)
2D	11.8	2	CS-SM3-7ADS / Annong 8455	CS-SM3-7ADS	(Ma et al., 2006a)
2DL	19.9	3	Veery / CJ 9306	CJ 9306	(Jiang et al., 2007b)
2DL	15.5	2	Veery / CJ 9306	CJ 9306	(Jiang et al., 2007a)
2DS	12.5	4	DH181 / AC Foremost	n/s	(Yang et al., 2005b)
2DS	11.1	1	DH181 / AC Foremost	DH181	(Yang et al., 2005b)
2DS	12.8	2	DH181 / AC Foremost	n/s	(Yang et al., 2005b)
2DS	12.1	2	Ning894037 / Alondra	Alondra	(Shen et al., 2003a)
3A	16.2	1	Frontana / Remus	Frontana	(Steiner et al., 2004)
3A	11.3	2	Nanda2419 / Wangshuibai	Wangshuibai	(Lin et al., 2004)
3AL	10	2	Arina / Forno	Forno	(Paillard et al., 2004)
3AS	11.8	1	DH181 / AC Foremost	AC Foremost	(Yang et al., 2005b)
3AS	13	2	F201R / Patterson	F201R	(Shen et al., 2003b)
3B	11.1	1 and 2	Cansas / Ritmo	Ritmo	(Klahr et al., 2007)
3B	11	1 and 2	Wangshuibai / Alondra 's'	Wangshuibai	(Jia et al., 2005)
3B	9.0 - 17.4	2	Nanda2419 / Wanghsuibai	Wangshuibai	(Lin et al., 2004)

Table 1.3 Continued

Chromosome	R2(%)d	Resistance Type	Mapping Population	Source of Resistance	Reference
3B	7.1-15.7	2	Wangshuibai / Alondra 's'	Wangshuibai	(Zhang et al., 2004)
3B	5.6 - 13.6	2	Nanda2419 / Wanghsuibai	Wangshuibai	(Lin et al., 2004; Li et al., 2008)
3B	13.7-23.8	2	Wangshuibai / Alondra 's'	Wangshuibai	(Zhang et al., 2004)
3B	13.3	2	Nanda2419 / Wanghsuibai	Wangshuibai	(Lin et al., 2004)
3B	9.6 - 19.6	4	Nanda2419 / Wanghsuibai	Wangshuibai	(Lin et al., 2006)
3BL	10.7	2	Huapei 57-2 / Patterson	Huapei 57-2	(Bourdoncle and Ohm, 2003)
3BS	92.6	3	CM-82036 / Remus	CM-82036	(Lemmens et al., 2005)
3BS	20.4 -37.8	2	CM-82036 / Remus	CM-82036	(Buerstmayr et al., 2003)
3BS	17	Unknown	Wangshuibai / Seri82	Wangshuibai	(Mardi et al., 2005)
3BS	11	3	Wuhan-1 / Maringa	Maringa	(Somers et al., 2003)
3BS	7.6 - 14.7	5	Veery / CJ 9306	CJ 9306	(Jiang et al., 2007b)
3BS	21.8 - 37.3	2	Wangshuibai / Wheaton	n/s	(Zhou et al., 2004)
3BS	60	2	Ning 7840 - Clark	Ning 7840	(Bai et al., 1999)
3BS	56.8	2	CM-82036 / Remus	CM-82036	(Buerstmayr et al., 2002)
3BS	42.5	2	Ning894037/Alondra	Ning894037	(Shen et al., 2003a)
3BS	41.6	2	Sumai-3 / Stoa	Sumai-3	(Anderson et al., 2001)
3BS	33.9	2	Wangshuibai / Wheaton	Wangshuibai	(Yu et al., 2008d)
3BS	33	2	W14 / Pion2684	W14	(Chen et al., 2006)
3BS	30.7	2	Veery / CJ 9306	CJ 9306	(Jiang et al., 2007a)
3BS	30.2	2	CS-SM3-7ADS / Annong 8455	CS-SM3-7ADS	(Ma et al., 2006a)
3BS	24.8	2	ND2603 / Butte 86	ND2603	(Anderson et al., 2001)

Table 1.3 Continued

Chromosome	R2(%)d	Resistance Type	Mapping Population	Source of Resistance	Reference
3BS	23.6	2	Huapei 57-2 / Patterson	Huapei 57-2	(Bourdoncle and Ohm, 2003)
3BS	22.5	3	Veery / CJ 9306	CJ 9306	(Jiang et al., 2007b)
3BS	17	2	Wangshuibai / Annong 8455	Wangshuibai	(Ma et al., 2006b)
3BS	16	2	Wangshuibai / Falat	Wangshuibai	(Najaphy et al., 2006)
3BS	15.4	2	Sumai-3 / Stoa	Sumai-3	(Waldron et al., 1999)
3BS[1]	13	2	Wuhan-1 / Maringa	Maringa	(Somers et al., 2003)
3BS	11	2	DH181 / AC Foremost	n/s	(Yang et al., 2005b)
3BS	13.6	1	Wangshuibai / Wheaton	Wangshuibai	(Yu et al., 2008d)
3BS	10	1	W14 / Pion2684	W14	(Chen et al., 2006)
3BS	10	2	W14 / Pion2684	W14	(Chen et al., 2006)
3BS	9.6 - 30.6	3	Wangshuibai / Wheaton	Wangshuibai	(Yu et al., 2008d)
3BS	44 - 50	Unknown	Ning 7840 / Clark	Ning 7840	(Yu et al., 2008a)
3BS	99	2	Sumai3*5 / Thatcher	Sumai3*5	(Cuthbert et al., 2006; Pumphrey et al., 2007)
3BS	99	2	HC374 / 3*98B69-L47	HC374 (Wuhan)	(Cuthbert et al., 2006)
3BS	29	4	W14 / Pion2684	W14	(Chen et al., 2006)
3BS	23	3	W14 / Pion2684	W14	(Chen et al., 2006)
3BSc	18	4	Ernie / MO 94-317	Ernie	(Abate et al., 2008)
3BSc	14	3	Ernie / MO 94-317	Ernie	(Abate et al., 2008)
3BSc	12.9	2	Ernie / MO 94-317	Ernie	(Liu et al., 2007)
3BSc	2.9 - 12.2	3	Wangshuibai / Wheaton	Wangshuibai	(Yu et al., 2008d)
3DL	11.2	1 and 2	Cansas / Ritmo	Cansas	(Klahr et al., 2007)

Table 1.3 Continued

Chromosome	R2(%)d	Resistance Type	Mapping Population	Source of Resistance	Reference
3DL	16.4	Fungal DNA	Arina / Riband	Riband	(Draeger et al., 2007)
4AL	10.1	Unknown	Arina / Forno	Arina	(Paillard et al., 2004)
4B	12.1 - 17.5	1	Nanda2419 / Wangshuibai	Wangshuibai	(Lin et al., 2006), 966
4B	10.4 - 23.4	4	Nanda2419 / Wangshuibai	Wangshuibai	(Li et al., 2008) 316
4BS	12	Unknown	Wuhan-1 / Maringa	Wuhan-1	(Somers et al., 2003)
4D	10.8	2	CS-SM3-7ADS / Annong 8455	CS-SM3-7ADS	(Ma et al., 2006a)
4DL	13.5	4	DH181 / AC Foremost	n/s	(Yang et al., 2005b)
4DL	12.5	1	DH181 / AC Foremost	DH181	(Yang et al., 2005b)
4DS	50.9	1 and 2	Spark/Rialto	Spark	(Srinivasachary et al., 2008)
4DS	31.5	1 and 2	Romanus / Pirat	Romanus	(Holzapfel et al., 2008)
4DS	29.2	1 and 2	History / Rubens	History	(Holzapfel et al., 2008)
4DS	16.3	1 and 2	Apache / Biscay	Apache	(Holzapfel et al., 2008)
4DS	12.9 - 23.9	AUDPC	Arina / Riband	Arina	(Draeger et al., 2007)
4DS	12.9	Fungal DNA	Arina / Riband	Arina	(Draeger et al., 2007)
4DS	11.4	4	Arina / Riband	Arina	(Draeger et al., 2007)
5A	16.6 - 27.0	1	Nanda2419 / Wangshuibai	Wangshuibai	(Lin et al., 2006)
5A	20	1	CM-82036 / Remus	CM-82036	(Buerstmayr et al., 2003)
5A	18	4	Ernie / MO 94-137	Ernie	(Abate et al., 2008)
5A	17.4	2	Ernie / MO 94-137	Ernie	(Liu et al., 2007)
5A	12.4	3	Wangshuibai / Annong 8455	Wangshuibai	(Ma et al., 2006b)

Table 1.3 Continued

Chromosome	R2(%)d	Resistance Type	Mapping Population	Source of Resistance	Reference
5A	10.9	2	CM-82036 / Remus	CM-82036	(Buerstmayr et al., 2002)
5A	10	3	Ernie / MO 94-137	Ernie	(Abate et al., 2008)
5AL	3.6 - 12.3	Unknown	Renan / Recital	Renan	(Gervais et al., 2003)
5AL	10.1 - 18.6	Unknown	Renan / Recital	Renan	(Gervais et al., 2003)
5AS	24	1	W14 / Pion2684	W14	(Chen et al., 2006)
5AS	16	2	W14 / Pion2684	W14	(Chen et al., 2006)
5AS	11.7	4	Arina / Riband	Riband	(Draeger et al., 2007)
5B	13.3	1 and 2	Wangshuibai / Alondra 's'	Wangshuibai	(Jia et al., 2005)
5B	10.8	1 and 2	Wangshuibai / Alondra 's'	Wangshuibai	(Jia et al., 2005)
5BL	20	1 and 2	Cansas / Ritmo	Cansas	(Klahr et al., 2007)
5BL	14.3	Unknown	Arina / Forno	Forno	(Paillard et al., 2004)
5DL	10.54	2	Chokwang / Clark	Chokwang	(Yang et al., 2005a)
5DL	7.7 - 10.0	3	Wangshuibai / Wheaton	Wangshuibai	(Yu et al., 2008d)
6A	13.9	1	Apache / Biscay	Apache	(Holzapfel et al., 2008)
6AL	19	Unknown	Dream / Lynx	Dream	(Schmolke et al., 2005; Haberle et al., 2007)
6AS	11.6	2	ND2603 / Butte 86	ND2603	(Anderson et al., 2001)
6B	19.5	2	Nanda2419 / Wangshuibai	Wangshuibai	(Lin et al., 2004)
6B	17.8	2	Nanda2419 / Wangshuibai	Wangshuibai	(Lin et al., 2004)
6BL	22.1	1 and 2	History / Rubens	Rubens	(Holzapfel et al., 2008)
6BL	9.9 - 14.8	AUDPC	Arina / Riband	Arina	(Draeger et al., 2007)
6BL	11.7	3	Arina / Riband	Arina	(Draeger et al., 2007)

Table 1.3 Continued

Chromosome	R2(%)d	Resistance Type	Mapping Population	Source of Resistance	Reference
6BL	10.2	Fungal DNA	Arina / Riband	Arina	(Draeger et al., 2007)
6BS	13.57	2	DH181 / AC Foremost	n/s	(Yang et al., 2005b)
6BS	10.0-27.0	2	Pelikan//Bussard/Ning8026	Ning8026	(Haberle et al., 2009)
6DL	22.1	Unknown	Arina / Forno	Arina	(Paillard et al., 2004)
7A	7.7-12.6	1 and 2	Wangshuibai / Alondra 's'	Wangshuibai	(Jia et al., 2005)
7AL	14.8	1 and 2	Arina / NK93604	NK93604	(Semagn et al., 2007)
7AL	16.9	AUDPC	Arina / Riband	Arina	(Draeger et al., 2007)
7AL	12.6	5	Arina / Riband	Arina	(Draeger et al., 2007)
7BL	16.5	AUDPC	Arina / Riband	Arina	(Draeger et al., 2007)
7BL	16.7	Fungal DNA	Arina / Riband	Arina	(Draeger et al., 2007)
7BL (later re-assigned to 3BS)	60	2	Ning 7840 / Clark	Ning 7840	(Bai et al., 1999)
7BS	11	1 and 2	Cansas / Ritmo	Cansas	(Klahr et al., 2007)
7BS	21	Unknown	Dream / Lynx	Dream	(Schmolke et al., 2005; Haberle et al., 2007)
7BS/5BL	11.0-24.0	2	Pelikan//Bussard/Ning8026	Ning8026	(Haberle et al., 2009)
7D	8.9 - 16.0	4	Nanda2419 / Wangshuibai	Wangshuibai	(Li et al., 2008)

[1] Erroneously reported as a Wuhan/Maringa derivative, later corrected as Wuhan / Nyubai (Somers et al., 2005).
n/s = not specified

study, the mapping populations Apache x Biscay, History x Rubens, and Romanus x Pirat were used (Holzapfel et al., 2008). High levels of resistance were seen in Romanus and History, resulting from several minor QTL and the absence of *Rht-D1b*. The semi-dwarf parents however contributed QTL of large effect, suggesting thecompensation for the negative effects of *Rht-D1b*. In all cases, the researchers suggested that the resistance was not due to plant height per se, but due to linkage or pleiotropy. (Holzapfel et al., 2008) suggest that the evidence from all the studies showed that the *Rht-D1b* allele had a pleiotropic effect on FEB resistance. This research also goes some way to explaining the susceptibility of UK cultivars, which in the majority of cases carry the *Rht-D1b* allele (Gosman et al., 2007). A common theme to emerge from these genetic studies is that resistance is not just contributed by the more resistant parent, sometimes the susceptible parents provide minor QTLs too (Singh et al., 1995; Waldron et al., 1999; Semagn et al., 2007) . The exact number of QTL involved and their location are a source of debate. Differences are likely to be seen for the following reasons:

1. Parents of the cross. The genetic background from the susceptible parent may mask the effect of the resistant.
2. Many different versions of the resistant cultivar Sumai 3 exist, each with a slightly different genetic background.
3. Different alleles of individual QTLs may be detected in different studies.
4. Resistance is a polygenic trait, so several different traits will be segregating in different crosses.
5. Infection and mycotoxin accumulation is highly influenced by the environment.
6. Variation in inoculation and scoring techniques.

(Waldron et al., 1999; Kolb et al., 2001; Yang et al., 2005a).

1.5 Other Examples of QTL Mediated Resistance

Disease resistance can be inherited quantitatively or qualitatively. Quantitative resistance involves many genes, each with differing contributions

to the final phenotype (resistance). In contrast, qualitative resistance is complete, based on single genes, as dictated by the gene-for-gene theory (see section 1.3). Quantitative resistance, although incomplete, is often considered more durable, avoiding the boom and bust cycle associated with single gene-mediated resistance which can be quickly overcome. Resistance to FEB is quantitative and mediated by QTL. Other diseases for which resistance is inherited quantitatively include the brown leaf spot diseases caused by *Septoria tritici* and *Pyrenophora tritici-repentis*, as well as the glume blotch pathogen *Phaeosphaeria nodorum* (Sip et al., 2005).

Common bean (*Phaseolus vulgaris* L.) resistance to common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* is quantitative and reportedly under the control of a few major genes (Silva et al., 1989; Liu et al., 2008). Three major QTL have so far been identified, but one of the current problems is the tight linkage with the deleterious *V* gene to one of the QTL, which confers a mottling appearance on coloured beans. Markers linked to the QTL are also linked to this gene and work is currently underway to identify new markers which break this linkage, for deployment in marker assisted selection (Liu et al., 2008) (discussed in further detail in Chapter 7).

Resistance loci for maize resistance to grey leaf spot (caused by *Cercospora zeae-maydis*) have been found on all 10 maize chromosomes (reviewed by Pozar et al. (2009)). Further QTL have been identified, but interactions between resistance loci and agronomic trait loci have in some cases been unfavourable. A consensus map of all published QTL has also been produced based on disease resistance to all published pathogens (Wisser et al., 2006).

1.6 Aims and Objectives

The aims of this project were to identify novel sources of resistance and explore the underlying resistance mechanisms, and are summarised as a flow chart in Figure 1.6. This was approached by evaluating the field reaction of newly obtained Chinese wheat material, which under Chinese field conditions was already shown to exhibit good resistance. The commercial cultivar Alsen was also included, which under USA conditions, exhibited resistance to FEB

and leaf rust (Oelke and Kolmer, 2005). Post harvest analysis determined the levels of mycotoxin contamination of the grain obtained from disease assessed ears. To determine the uniqueness of the genotypes, genotyping was done using previously published markers for three of the major QTLs. The infection biology of FEB was explored using transgenic strains of the sequenced *F. graminearum* isolate PH-1 in the appropriate containment facilities at Rothamsted Research. Strains expressing the reporter gene GUS (β-glucuronidase) under either a constitutive promoter, to quantify fungal biomass, or the *tri5* promoter, to quantify the expression of *Tri5* and therefore giving an indication of the induction of DON biosynthesis, were used. A leaf bioassay previously developed by the Rothamsted Research group was also explored further as a potential method for the screening of wheat genotypes for resistance.

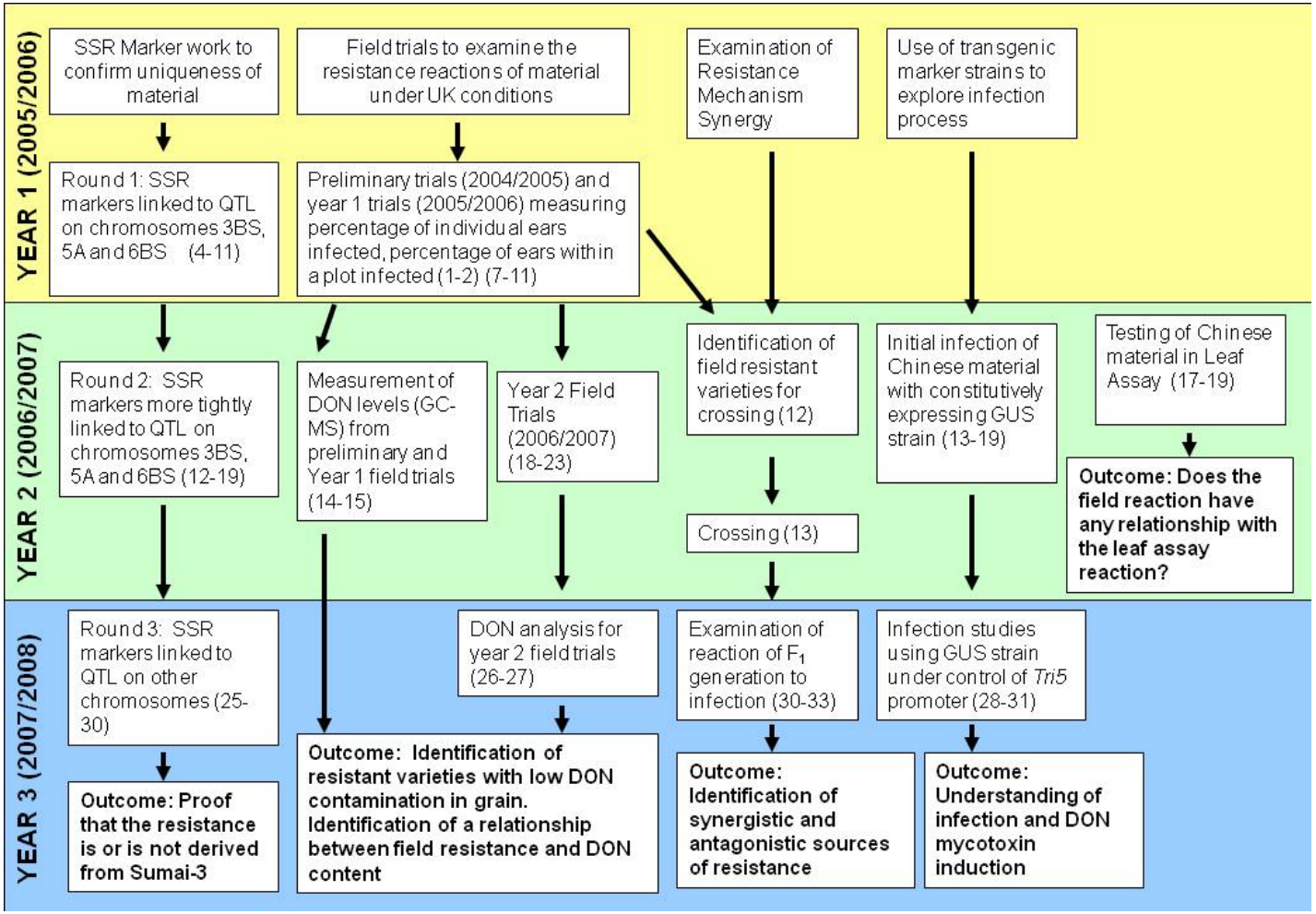


Figure 1.6 Flow chart summarising the aims and objectives for this project. Projected time scales are included in months after start of project

CHAPTER 2: MATERIALS AND METHODS

2.1 Growth of Fusarium Cultures

Strains used in these studies:

- *Fusarium culmorum* UK-99 (FGSC 10436): used for field trials (see Chapter 4) and the leaf bioassay (see Chapter 6).
- *F. culmorum* 98-11 (Daudi, 2007): used for the leaf bioassay (see Chapter 6).
- *F. graminearum* Fg602.10 (RF025): used for field trials (see Chapter 4).
- *F. graminearum* Fg576.1 (RF026): used for field trials (see Chapter 4).
- *F. graminearum* PH-1 (NRRL 31084): used for glasshouse infection studies (see Chapter 5) and leaf bioassay (see Chapter 6).
- *F. graminearum* MU102 : transgenic strain of *F. graminearum* PH-1 carrying a deleted copy of the *Tri5* gene. Used for the leaf bioassay (see Chapter 6)
- *F. graminearum* G3: transgenic strain of *F. graminearum* PH-1 carrying the GUS gene under a constitutive promoter. Used for glasshouse infection studies (see Chapter 5).
- *F. graminearum* GP17: transgenic strain of *F. graminearum* PH-1 carrying the GUS gene under control of the *Tri5* gene promoter. Used for glasshouse infection studies (see Chapter 5).

(a)Synthetic Nutrient-deficient Agar (SNA)

(Used for maintaining stocks in preparation for bulking of conidia)

KH_2PO_4	1.0g
KNO_3	1.0g
$MgSO_4.7H_2O$	1.0g
KCl	0.5g
Glucose	0.2g
Sucrose	0.2g
Agar Number 3 (Oxoid Ltd, Basingstoke, UK)	20.0g

Distilled Water	1000ml

(b) Potato Dextrose Agar (PDA)

(Used to bulk conidia of *F. culmorum*)

Synthetic Potato Dextrose Agar (Oxoid Ltd, Basingstoke, UK)	39.0g
Distilled Water	1000 ml

(c) Potato Dextrose Broth (PDB)

(Used in the preparation of cultures for long term storage)

Potato dextrose broth (Sigma)	24.0g
Distilled Water	1000 ml

(d) Mung Bean Liquid Media

(Used to bulk conidia for *F. graminearum* strains)

40.0 g mung beans were added to1000 ml distilled water, and boiled for approximately 10 minutes until the first seed coats appeared to loosen. The beans were extracted from the liquid by straining through miracloth (Calbiochem®), and the remaining liquid was autoclaved prior to inoculation (Bai et al., 2000).

(e) TB3

(Used to bulk conidia for *F. graminearum* strains for the leaf bioassay experiments, chapter 6)

Yeast extract	3.0g
Peptone	3.0g
Sucrose	200.0g
Water	1000 ml

Long Term Storage

Fusarium isolates were stored at -80 ºC for long term storage. The best method for storage was found to be on soil stocks (Dr. M. Urban, Rothamsted Research, pers. comm.). Soil was autoclaved before aliquoting into 1.8 ml cryotubes (nunc^{TM}, Fisher Thermofisher Scientific, Roskilde, Denmark) and autoclaving once more. A 200 µl aliquot of potato dextrose broth was added before an agar plug of approximately 0.25 cm^2 was added to soil stocks. The stocks were incubated at 20 ºC for 10 days before storing at -80 ºC.

Growth and Maintenance of Cultures

Isolates of *F. culmorum* and *F. graminearum* were maintained on 9cm SNA plates prior to conidia generation. Plates were placed in incubators (manufactured in-house at Rothamsted Research) at room temperature, illuminated using near UV and white light. After 7-10 days conidia were produced. For large scale production of conidia for inoculation, PDA plates and mung bean liquid media were employed. PDA plates were used to generate high concentrations of conidia from *F. culmorum* isolates. Conidia from 7-10 day old SNA cultures were gently dislodged using 1.5ml sterile distilled water with a sterile disposable spreader. A 200 µl aliquot of the conidia suspension was spread onto a fresh 9 cm PDA plate. The plates were left unsealed in the incubator for 4 days. Typically, plates developed a deep red pigmentation, and conidia were generated in high concentrations for the preparation of inocula as described previously.

Conidia from isolates of *F. graminearum* were generated using flasks of mung bean liquid media inoculated with 4-6 1cm^2 agar plugs from the margin of 7 day old SNA cultures (see SNA cultures) before incubating at 27 ºC at 220 rpm for 5 days (Gallenkamp Orbital Incubator, Loughborough, UK). Conidia were extracted from the liquid culture by centrifugation (Sigma 3K10 Laborzentrifugen, Sigma). For most applications, conidia were filtered through a double layer of miracloth to remove mycelial fragments. For the bulking of conidia of *F. graminearum* isolates for the leaf bioassay, 2ml of TB3

was added to an SNA culture which was at least seven days old, spread, and the excess removed. This was later refined to the addition and spreading of 200 µl to an SNA culture at least seven days old without removal of the excess. Spore concentrations were determined using a haemocytometer (Brightline, Hausser Scientific, Horsham, PA, USA). Two droplets of 15 µl were placed on the haemocytometer and the number of conidia present on the slide within the largest square counted. This was carried out for both segments of the haemocytometer, and the mean was taken for the two samples. This mean was equal to:

(number of conidia multiplied x 10^4) ml $^{-1}$

2.2 Growth of Wheat Plants

Wheat seeds were grown in Rothamsted Prescription Mix compost (Petersfield Products, Leicester, UK), which consisted of 75% medium grade peat, 12% screen sterilised loam, 3% medium grade vermiculite and 10% grit (6mm screened, lime-free). Osmocote Exact 3/4 Month slow release fertiliser (Scotts UK Professional, Ipswich, Suffolk) was added at 3.5 kg per m^3, together with 0.5 kg PG mix per m^3 (Hydro Agri (UK) Ltd., Immingham, UK). Lime was added to pH 5.5 -6.0 and Vitax wetting agent at 200ml per m^3 (Vitax Ltd, Coalville, UK). Seedlings were vernalised at 5 ºC for 8 weeks before being propagated under controlled environment conditions with a daily temperature regime of 20 ºC day, 18 ºC night, 16 hour day for non-containment glasshouses and 14 hour day for containment facilities. SON-T 400W sodium lamps were used with a fluence level of 300 µmol m^2 sec^{-1}. This regime applied to all plant material except for those grown under field conditions (Chapter 4).

2.3 Statistics

All data was analysed using GenStat v11statistical software (Payne et al., 2005). Specific analyses are detailed in each individual chapter.

2.4 Photography

Photographs were taken using the Nikon digital cameras D40X and D80 (Nikon Corporation, Japan).

CHAPTER 3: MOLECULAR CHARACTERISATION OF WHEAT GERMPLASM

3.1 Introduction: Choice of Wheat Genotypes and Markers

The wheat genotypes selected for this project were of diverse origin and different levels of resistance to FEB. To date, Sumai 3 is the best known source of resistance to FEB and as such is used in breeding programmes worldwide (McCartney et al., 2004). In this project, Sumai 3 was used as the resistant control. Ning 7840 and Alsen, derivatives of Sumai 3 which show good levels of resistance to FEB (Bai et al., 1999; Oelke and Kolmer, 2005; Frohberg et al., 2006) were also included. The genotypes China 1-8 were donated to this project as part of a collaboration between Rothamsted Research and Dr. Binjie Gan (Anhui Academy of Agriculture Sciences, Hefei, China). Field trials in China over many years demonstrated that these genotypes displayed high levels of resistance under natural and artificially induced FEB epidemic conditions. Pedigree information from China indicated that China 1 and China 6 have some parents in common, China 2 is a version of Sumai 3, China 3 is distinct from the other genotypes, China 4 is different to China 1 and China 6, China 5 and China 8 have some parents in common, and China 7 is a variant of China 8. Full pedigree information is given in the appendix A1. Other genotypes were included in this analysis for comparison and also used in the subsequent field trials (Chapter 4). CIMMYT 1 and CIMMYT 2 are genotypes originating from the CIMMYT wheat breeding program received via a Monsanto wheat breeder, and USA1 is the assigned name for the American hard red winter wheat Jagger (Sears et al., 1997). In previous field trials at Rothamsted Research, the genotypes CIMMYT 1, CIMMYT 2, and USA1 had demonstrated good levels of FEB resistance (Daudi, 2007). Bobwhite is one of a series of sister genotypes from the Bobwhite series (www.cimmyt.org) which were released in the early 1970s, and was included as a susceptible control. The genotype is used as a control throughout the Rothamsted Fusarium Group. Consort is a FEB susceptible high yielding British winter wheat originating from the PBI Cambridge.

The aim of this PhD was to identify novel sources of resistance to FEB. The Chinese genotypes were the main focus of the project and it was important to firstly determine genetic novelty to ensure there was no Sumai 3 in the pedigree, and secondly to examine the resistance under UK field conditions (see Chapter 4). Three loci of interest have been repeatedly detected as contributing to FEB resistance on the chromosomes 3BS, 5A, and 6BS (see Chapter 1 section 1.4 for more details). Based on this previous work, five SSR markers and two STS markers were selected to detect polymorphisms between Sumai 3 and the other genotypes at the three QTL. Markers *Xgwm533*, *XSTS3B-138*, *STS3B-142* and *Xgwm493* were selected for chromosome 3B, *Xgwm304* for chromosome 5A, and *Xgwm133* and *Xgwm644* for chromosome 6B (Figure 3.1). Haplotyping at these loci is considered a good method to determine novel sources of resistance and markers previously mapped close to the FEB resistance QTL have been used to screen material that demonstrate a good level of resistance to determine the presence or absence of known QTL (McCartney et al., 2004; Yang et al., 2006; Yu et al., 2006). Haplotyping also provides a method of detecting resistance in genotypes which may have the same resistance but have different underlying resistance genes (Bai et al., 2003). This would allow a breeder to select for different resistance mechanisms and therefore provide more durable resistance.

In addition to these main resistance loci, the dwarfing gene loci *Rht-B1* and *Rht-D1* have been linked to FEB susceptibility (detailed in section 1.4 Chapter 1) (Hilton et al., 1999; Draeger et al., 2007; Holzapfel et al., 2008; Srinivasachary et al., 2008; Voss et al., 2008). The loci *Rht-B1* and *Rht-D1* are located on chromosomes 4BS and 4DS, respectively (Srinivasachary et al., 2009). The wild-type allele *Rht-D1a* is associated with resistance, the *Rht-D1b* mutant allele linked with susceptibility. The effect of the *Rht-B1* locus on resistance is more complicated, with an apparent increase in susceptibility associated with *Rht-B1b* under field conditions (spray), but an increase in resistance under polytunnel (point inoculated) (Srinivasachary et al., 2009). Taken together, this data suggests that the *Rht-B1b* and *Rht-D1b* alleles

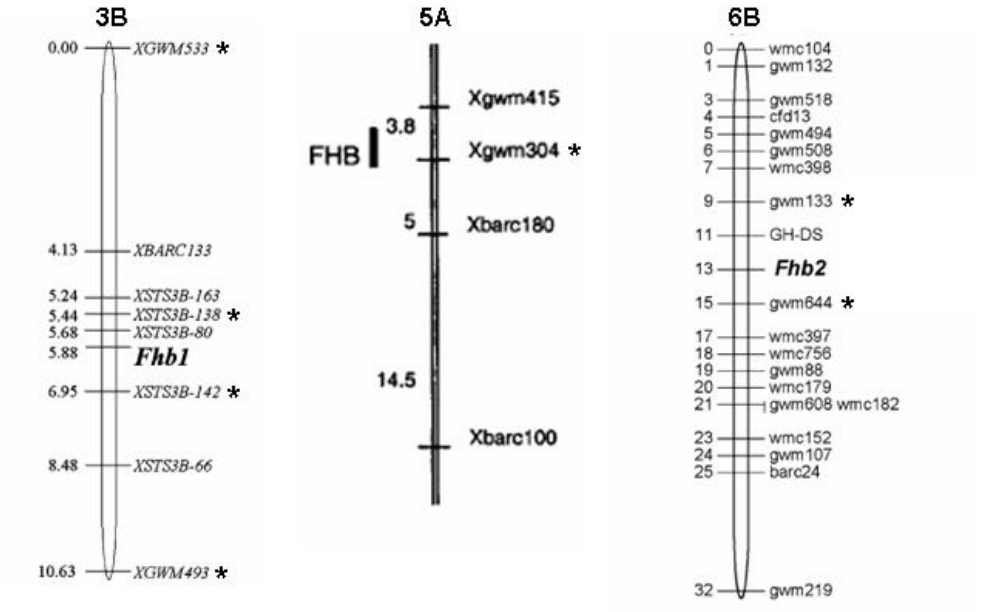


Figure 3.1 Genetic maps of parts of wheat chromosomes 3B, 5A, and 6B taken from Cuthbert *et al*. (2006), Shen *et al*. (2003) and Cuthbert *et al* (2007) respectively show the location of *Fhb1* and *Fhb2* loci, and the FHB QTL on chromosome 5A. Asterisks indicate markers used in this project.

confer susceptibility to spray inoculation (type I resistance), and that the *Rht-B1b* allele confers resistance to point inoculation (type II resistance). Markers previously described for these loci were used (Ellis et al., 2002).

Diversity Array Technology (DArT) marker analysis was also employed to distinguish between the genotypes at the whole genome level. The DArT system was first developed in rice and in just eight years has already been applied to 52 species (Jaccoud et al., 2001), www.diversityarrays.com). DArT markers are generated when the genomic DNA of a selection of genotypes representing a particular species, in this case wheat, is digested with restriction enzymes. For the development of the wheat DArT array, 13 Australian-grown wheat cultivars were pooled, and digested using *Pst*I and *Mse*I. Digestion of the DNA using these enzymes would yield many different sized bands which would be difficult to deal with in a species as complex as wheat. To reduce the complexity of the genome, the fragments were ligated to a *Pst*I adapter, and then amplified by PCR. Only the small fragments were amplified due to the limitations in PCR for amplifying large fragments, thereby reducing the number of fragments. This subset was termed the genomic representation of a genotype and each of the fragments is a DArT marker. The representation was cloned, spotted on a microarray and denatured. DNA from a genotype of interest was digested and cloned in the same way before being labelled with cy3-dUTP, giving another genomic representation. Denatured labelled fragments were then hybridised with the array, the resulting microarray signal indicating the presence or absence of a DArT marker in the genotype of interest. This was repeated using 411 wheat lines to identify markers which were polymorphic, in order to produce an improved array which was enriched for polymorphic markers. Markers from a selection of under-represented wheat genotypes were also included in this improved array. A linkage map integrating DArT markers and SSR, RFLP, and AFLP markers was generated using the Cranbrook x Halberd doubled haploid mapping population (Akbari et al., 2006) on the improved DArT array. This demonstrated that DArT markers map to all 21 chromosomes except 4D. The major advantage of this method is that many loci can be scored

simultaneously, in contrast to the PCR-based SSR and STS markers which would require multiple assays (Jaccoud et al., 2001), www.triticarte.com.au).

3.2 Materials and Methods

Isolation of DNA and Amplification of SSRs

Wheat plants were grown under glasshouse conditions to the two leaf stage before DNA extraction was carried out following a modified CTAB extraction protocol (Saghai Maroof et al., 1984). Equal amounts (1 cm^2) of plant material were used from four different plants from each of eleven genotypes (Table 3.1). The plant tissue was added to 200 µl of extraction buffer (0.14M D-sorbitol, 0.22M TRIS-HCl pH 8.0, 0.022M EDTA pH 8.0, 0.8M NaCl, 0.8% CTAB, 1% lauroylsarcosine) and ground to near homogeneity using a plastic grinder. The homogenates were incubated at 65 ºC for 5-10 minutes before 200 µl chloroform/*iso*-amylalcohol (24:1) was added. The tube was vortexed for 1-2 minutes. Samples were spun at 13,500 rpm and the upper phase containing the DNA was removed to a fresh tube. Two hundred microlitres of iso-propanol was mixed with the phase and the DNA was allowed to precipitate for 10-15 mins at room temperature. Samples were spun at 13,500 rpm for 10-15 minutes and the supernatant was removed. Seven hundred and fifty microlitres of 70% ethanol was added before briefly vortexing the sample and spinning at 13,500 rpm for 5 minutes. The supernatant was removed and the DNA pellet was dried at room temperature before being resuspended in sterile deionised water. DNA quality was checked by running a 5 µl sample through a 0.8% agarose gel (Sigma) stained with ethidium bromide and visualised using the Genesnap version 6.03 image acquisition software (Synoptics Ltd). This method was used in studies from year 1 only. Concentration of DNA was determined using the Eppendorf Biophotometer (Eppendorf, Cambridge, UK).

SSR primers were obtained based on sequences detailed in Röder *et al*. (1998). Initially in 2006, four SSR markers were used for the haplotyping; *Xgwm533*, *Xgwm493*, *Xgwm304*, and *Xgwm644* (Table 3.2). PCR reactions

Table 3.1 Wheat genotypes used in the haplotype analyses and field trials

Field Code (V)	Genotype	Preliminary FEB Haplotype Analysis	Final FEB Haplotype Analysis	*Rht-B1*, *Rht-D1* and DArT Analysis	Field Trials
2	Petrus			✓	✓
6	Consort		✓	✓	✓
7	Rialto			✓	✓
10	Frelon			✓	✓
11	Sumai 3	✓	✓	✓	✓
12	Ning 7840		✓	✓	✓
13	Wuhan			✓	✓
14	USA1		✓	✓	✓
15	Bobwhite	✓	✓	✓	✓
16	Gottingen 2			✓	✓
17	Gottingen 3			✓	✓
18	CIMMYT 1		✓	✓	✓
19	CIMMYT 2		✓	✓	✓
22	Paragon			✓	✓
23	Bobwhite			✓	✓
24	China 3	✓	✓	✓	✓
25	China 4	✓	✓	✓	✓
26	China 7	✓	✓	✓	✓
27	China 6	✓	✓	✓	✓
28	China 1	✓	✓	✓	✓
29	Alsen	✓	✓	✓	✓
30	China 2	✓	✓	✓	✓
31	China 5	✓	✓	✓	✓
32	China 8	✓	✓	✓	✓

Table 3.2 Primer sequences, repeats, and expected band sizes for the FEB SSR and STS markers used in the haplotyping of wheat genotypes

Marker	Forward Primer	Reverse Primer	Repeat	Expected Band Size for Sumai 3 (bp)	Reference
Xgwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	(CT) 18 (CA) 20	141	(Roder et al., 1998; Cuthbert et al., 2006)
STS3B-138	CAAGATCAAGAAGGCCAAGC	AGGTACACCCCGTTCTCGAT		355	(Roder et al., 1998; Cuthbert et al., 2006)
STS3B-142	CGAGTACTACCTCGGCAAGC	CATAGAATGCCCCGAAACTG		156	(Roder et al., 1998; Cuthbert et al., 2006)
Xgwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	(CA) 43	195	(Roder et al., 1998; Cuthbert et al., 2006)
Xgwm304	ACGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	(CT) 22	234	(Roder et al., 1998; Cuthbert et al., 2007)
Xgwm133	ATCTAAACAAGACGGCGGTG	ATCTGTGACAACCGGTGAGA	(CT) 39	120	(Roder et al., 1998; Cuthbert et al., 2007)
Xgwm644	GTGGGTCAAGGCCAAGG	AGGAGTAGCGTGAGGGGC	(GA) 20	206	(Roder et al., 1998; Cuthbert et al., 2007)
Xgwm389	ATCATGTCGATCTCCTTGACG	TGCCATGCACATTAGCAGAT	(CT) 14 (GT) 16		(Roder et al., 1998)
Xwmc054	TATTGTGCAATCGCAGCATCTC	TGCGACATTGGCAACCACTTCT	(GT) 8		http://wheat.pw.usda.gov
Xwmc078	AGTAAATCCTCCCTTCGGCTTC	AGCTTCTTTGCTAGTCCGTTGC	(CA) 18		http://wheat.pw.usda.gov
Xwmc231	CATGGCGAGGAGCTCGGTGGTC	GTGGAGCACAGGCGGAGCAAGG	(GA) 10		http://wheat.pw.usda.gov

(20 µl) contained 25ng DNA, 10 mM each dNTP, 7.5 pmol each primer, 0.5 mM $MgCl_2$, 1.25 U *Taq* DNA Polymerase (Invitrogen), 2µl 10 x PCR Buffer minus Mg (Invitrogen), 0.05% W-1 (Invitrogen). PCR amplifications were carried out on a GeneAmp® PCR System 9700 (GE Applied Biosystems) and DNA Engine TETRAD™ 2 Peltier Thermal Cycler (Bio-Rad) machines. The cycling conditions were as follows: denature at 94 ºC for 3 minutes, followed by 45 cycles of: 94 ºC for 1 min, 60 ºC (*Xgwm533*, *Xgwm493*, *Xgwm644*) or 55 ºC (*Xgwm304*) for 1 min, and 72 ºC for 2 min. A final extension step followed at 72 ºC for 10 min. PCR products (size range 100-220 bp) were visualised on a 1.5% NuSieve® 3:1 Agarose : 1.5% MetaPhor® Agarose gel (Cambrex Bio Science Rockland Inc., USA) stained with ethidium bromide (10 µg/µl) and viewed using a UV transilluminator.

Genotypes were scored by comparing banding patterns to those obtained for Sumai 3 (positive control) and Bobwhite (negative control) on a basis of "same (✓)" or "different (x)" size PCR product. Further modifications were made when the experiments were continued in 2008. Additional wheat genotypes were added (Table 3.1) and the markers *STS3B-138*, *STS3B142* and *Xgwm133* (Table 3.2) were used in addition to the original four. In year 3, DNA was isolated from tissue from two leaf stage wheat seedlings grown under standard glasshouse conditions using the GenElute™ Plant Genomic DNA Miniprep kit (Sigma) according to the manufacturer's instructions. DNA quality was checked by running a 5 µl sample through a 0.8% agarose gel (Sigma) stained with ethidium bromide and visualised using the Genesnap version 6.03 image acquisition software (Synoptics Ltd). Concentration of DNA wasdetermined using the Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Primers were labelled with the infrared dye DY-681 and DY-781 (Biomers.net GmbH, Germany). PCR reactions (10 µl) contained 30ng template DNA, 5 µl 2 x Promega mastermix, and 1 pmol each primer. PCR amplifications were carried out on DNA Engine TETRAD™ 2 Peltier Thermal Cycler (Bio-Rad) and G-STORM™ GS4 (Gene Technologies Ltd) machines. The cycling conditions were as follows: denature at 94 ºC for 2 minutes, followed by 35 cycles of: 94 ºC for 20 secs,

50 ºC (*Xgwm304*, *STS3B-138*), 55 ºC (*STS3B-142*) or 60 ºC (*Xgwm493*, *Xgwm644*, *Xgwm533*, *Xgwm133*) for 30 secs, and 72 ºC for 1 min. A final extension step followed at 72 ºC for 5 min. PCR products were diluted with loading dye (36.75% v/v deionised formamide, 0.1 M EDTA pH 8, 0.09 mg/ml bromophenol blue, 62.5% v/v ml deionised water) and denatured at 85 ºC for 5 mins before being visualised on a SequalGel XR® acrylamide gel, made according to the manufacturers instructions. PCR products were run through the gel using a LI-COR 4300 DNA Analyser, New Life Science Products Ltd, USA). A 25 minute pre-run was carried out prior to the electrophoresis of PCR products at 1500 V for 15 minutes. Electrophoresis was carried out at 1500 V for 90 minutes.

Four additional SSR markers were used to determine the haplotype of the short arm of chromosome 3B: *Xgwm389*, *Xwmc54*, *Xwmc78*, and *Xwmc231* (Table 3.2). PCR amplification was carried out as before with the annealing temperatures 50 ºC (*Xwmc54* and *Xwmc78*) and 60 ºC (*Xgwm389* and *Xwmc231*) and products visualised using the LI-COR system.

Rht-B1 and *Rht-D1* alleles were determined by Carlos Bayon, Rothamsted Research, using markers previously described by Ellis et al. (2002), Table 3.3. DArT marker analysis was carried out by Triticarte, Australia (www.triticarte.com.au) based on the method described above. The genotypes under investigation in this project were analysed using an array which successfully used 1857 markers covering all 21 wheat chromosomes. The genotypes were scored as '0' or '1' indicating the presence or absence of a particular marker. Clustering analysis based on the results was carried out by Dr. Hai-Chun Jing, Rothamsted Research, using principal coordinate analysis on GenStat v11 (Payne et al., 2005).

3.3 Results

Sixteen of the 24 wheat genotypes used throughout this PhD were haplotyped with a selection of markers linked to QTL conferring resistance to FEB. Preliminary studies done in year 1 had used the four markers *Xgwm493*, *Xgwm533*, *Xgwm644* and *Xgwm304* (only data for *Xgwm493*

Table 3.3 Primer sequences for the *Rht* allele analysis (carried out by C. Bayon, Rothamsted Research)

Marker	Forward Primer	Reverse Primer	Reference
Rht-B1b	GGTAGGGAGGCGAGAGGCGAG	CATCCCCATGGCCATCTCGAGCTA	(Ellis et al., 2002)
Rht-D1b	CGCGCAATTATTGGCCAGAGATAG	CCCCATGGCCATCTCGAGCTGCTA	(Ellis et al., 2002)
Wild Type B	GGTAGGGAGGCGAGAGGCGAG	CATCCCCATGGCCATCTCGAGCTG	(Ellis et al., 2002)
Wild Type D	CGCGCAATTATTGGCCAGAGATAG	GGCCATCTCGAGCTGCAC	(Ellis et al., 2002)

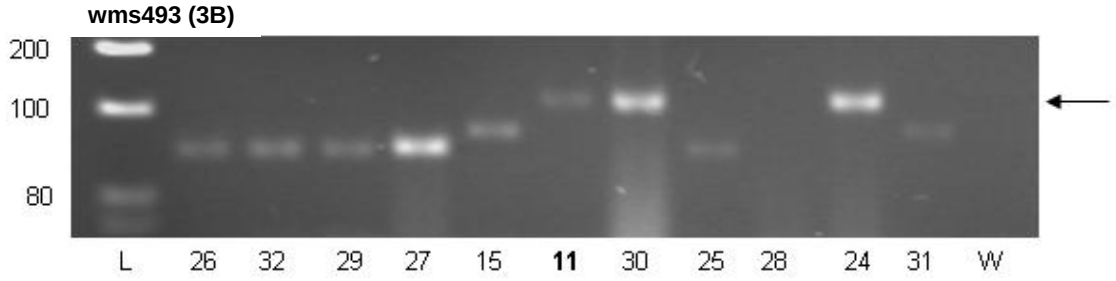


Figure 3.2 Alleles of the marker wms493 (3B) using a conventional agarose gel with ethidium bromide staining. Genotypes are numbered according to Table 3.1. Size ladder (L) is given in base pairs. Sumai 3 (11) is shown in bold and the arrow indicates the diagnostic band size for Sumai 3.

shown, Figure 3.2) and a standard agarose gel with ethidium bromide staining. Polymorphism between genotypes was seen in most cases but unsatisfactory resolution of products prompted the use of a more powerful system for detection of small polymorphisms. Acrylamide gels are better suited to separate PCR products differing only slightly in size. The LI-COR DNA analyser can detect single base pair differences (Comai et al., 2004) and so primers for the original markers as well as some additional markers were end labelled with fluorescent IR-dyes for use in this system. In the interim period between the preliminary studies and the more detailed analysis in year 3, the QTL on chromosomes 3BS and 6BS were resolved as Mendelian traits and the chromosome regions were saturated with markers (Figure 3.1). Therefore the STS markers STS3B-138 and STS3B-142 and the SSR marker *Xgwm*133 were included, along with some of the other wheat genotypes now of further interest as a result of the 2005/2006 and 2006/2007 field trial results (see Tables 3.1 and 3.2).

The marker alleles for Sumai 3 (resistant) and Bobwhite (susceptible) are given in Figure 3.3. Polymorphism with each marker between the resistant and susceptible genotypes was demonstrated using the LI-COR system. A wider survey of the genotypes is given in Figure 3.4. This shows the marker alleles from the different genotypes with the seven markers. PCR products were scored as either the same or different to the banding pattern for Sumai 3. The arrow in each figure indicates the position of the diagnostic band. The overall results are summarised in Table 3.4. Only one of the genotypes tested had the same haplotype as Sumai 3 across all loci, which was China 2 (Table 3.4). This was the source of Sumai 3 provided by Dr. Binjie Gan and acted as a good internal control. Nine of the genotypes had between one and five marker alleles in common with Sumai 3 and six genotypes had no marker alleles in common with Sumai 3. In most cases, where genotypes have marker alleles in common with Sumai 3, only one of the flanks of a particular QTL was in common. For example, six genotypes had marker alleles in common with Sumai 3 at *Xgwm*133, but not on *Xgwm644*, which suggests that this FEB resistance QTL is not entirely present in these genotypes. The 3BS QTL (*Fhb1*) was detected

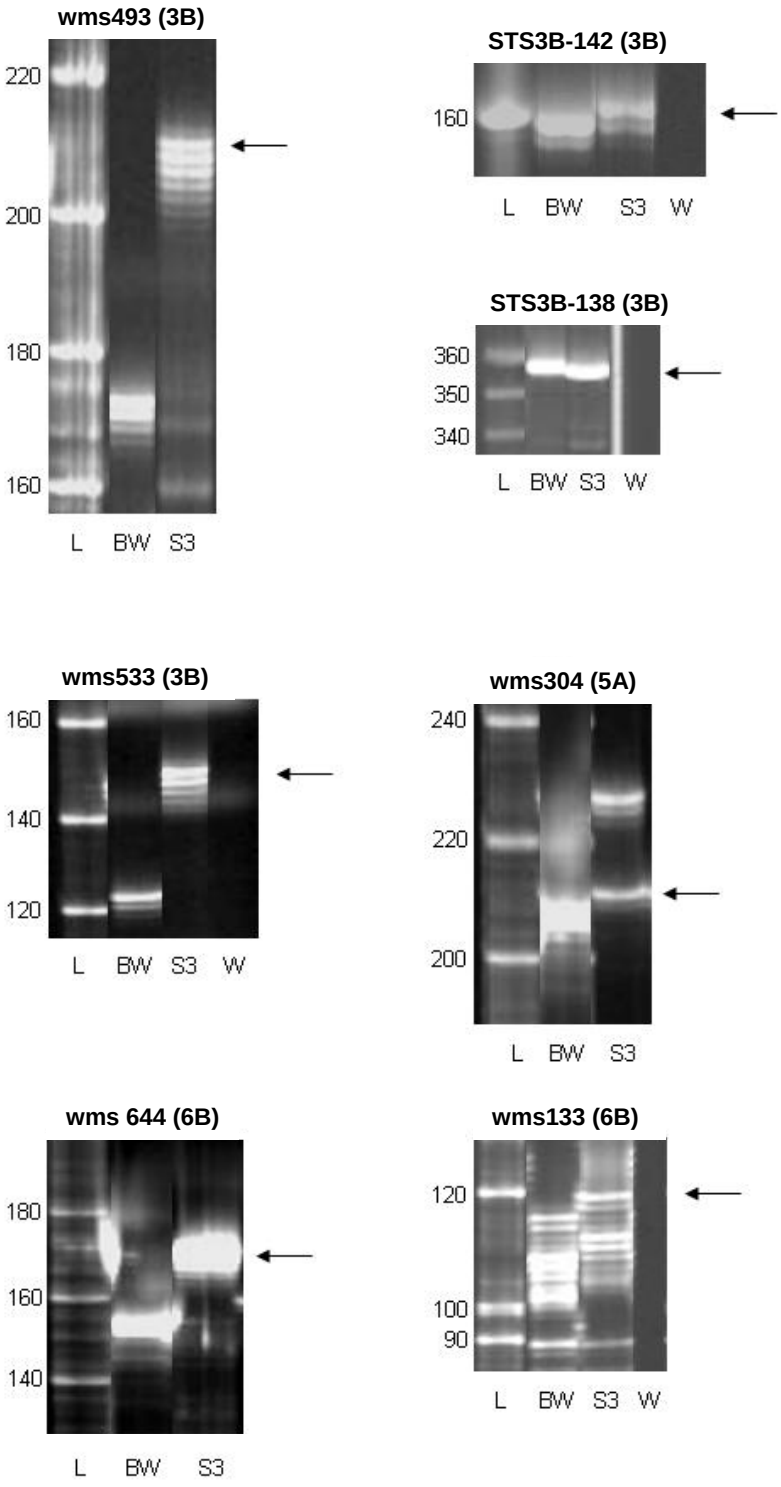


Figure 3.3 PCR product polymorphism demonstrated on polyacrylamide gels between Bobwhite (BW) and Sumai 3 (S3). Marker numbers are given on the top left of each picture. When primers were previously untested, water controls (W) were also used in the PCR. Size ladders are given in bp to the left of the images (L). Arrows indicate the band sizes obtained for Sumai 3 for comparison.

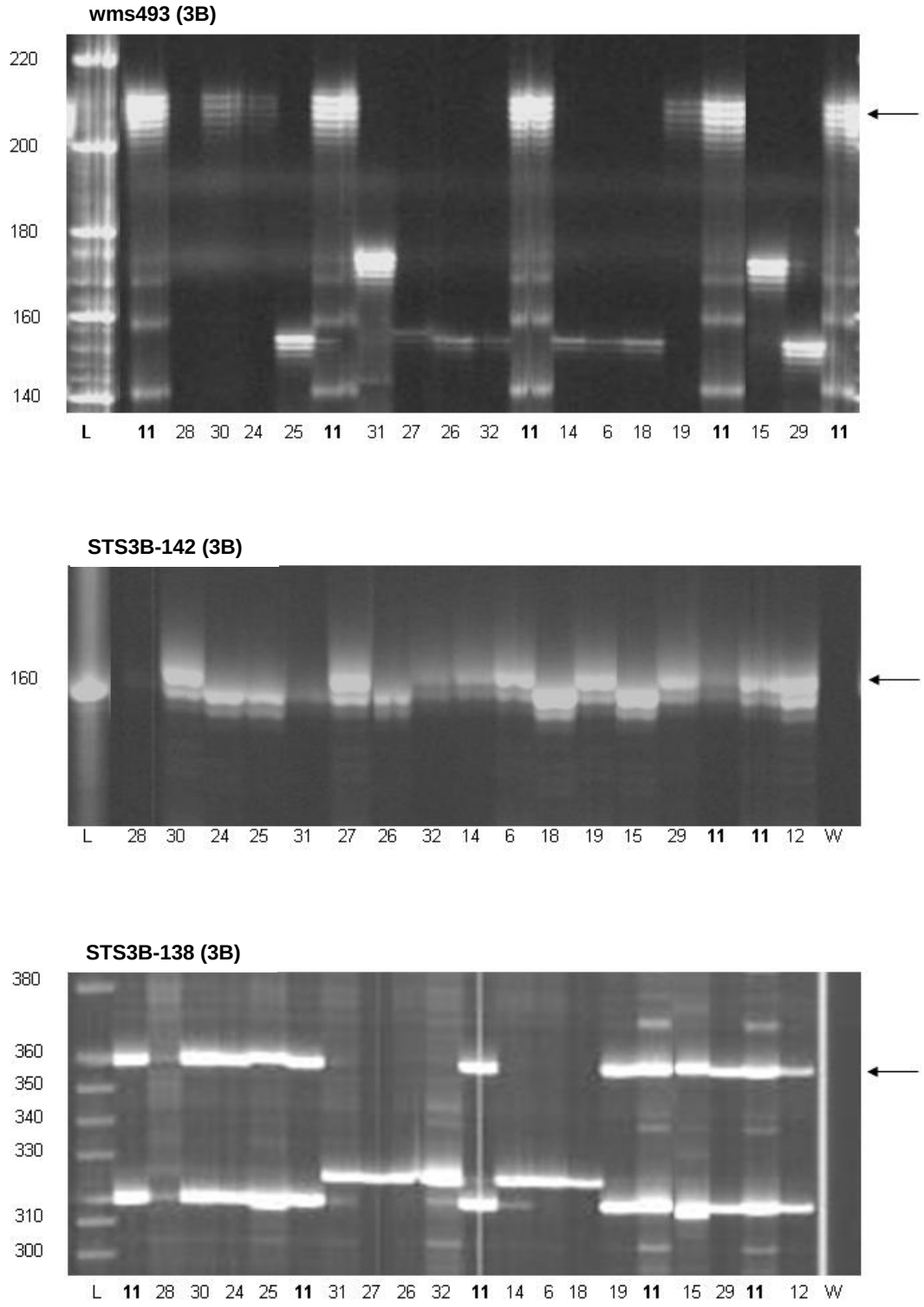


Figure 3.4 Alleles of the markers wms493, STS3B-14, STS3B-138 (this page), wms533, wms304, wms644, and wms133 (next page). Genotypes are numbered according to Table 3.1. Size ladder (L) is given in base pairs. Sumai 3 (11) is highlighted and arrows indicate the diagnostic band size for Sumai 3.

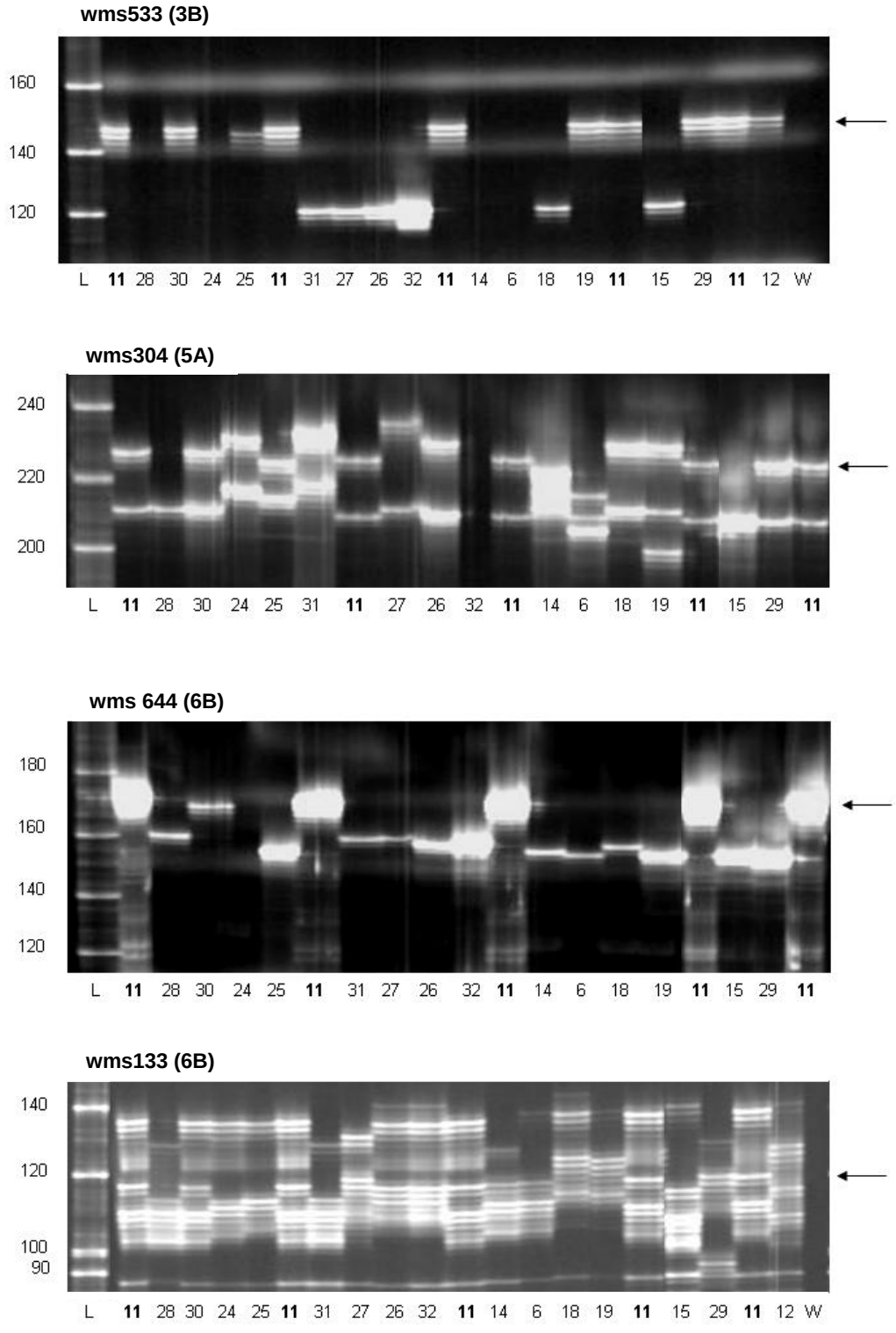


Figure 3.4 Alleles of the markers wms493, STS3B-14, STS3B-138 (previous page), wms533, wms304, wms644, and wms133 (this page). Genotypes are numbered according to Table 3.1. Size ladder (L) is given in base pairs. Sumai 3 (11) is highlighted and arrows indicate the diagnostic band size for Sumai 3.

Table 3.4 Microsatellite allele comparison between Sumai 3 and a selection of wheat genotypes used throughout this project

Genotype	Chromosome 3BS				Chromosome 5A	Chromosome 6B	
	wms 493	STS3B-142	STS3B-138	wms 533	wms 304	wms 644	wms133
China 1	N	N	N	N	x	x	x
China 2	✓	✓	✓	✓	✓	✓	✓
China 3	✓	x	✓	N	x	N	x
China 4	x	x	x	x	x	x	x
China 5	x	x	x	x	x	x	x
China 6	x	✓	x	x	x	x	✓
China 7	x	x	x	x	x	x	✓
China 8	x	✓	x	x	x	x	✓
USA1	x	✓	x	N	x	x	✓
Consort	x	✓	x	N	x	x	✓
CIMMYT 1	x	x	x	x	x	x	x
CIMMYT 2	✓	✓	✓	✓	x	x	x
Bobwhite	x	x	x	x	x	x	x
Alsen	x	✓	✓	✓	✓	x	✓
Sumai 3	✓	✓	✓	✓	✓	✓	✓
Ning 7840	✓	✓	✓	✓	x	x	x

N = Null allele; ✓ = allele same as Sumai 3; x = allele different to Sumai 3

completely at all four marker loci in CIMMYT 2 and Ning 7840. Alsen also had three out of four marker alleles in common with Sumai 3, and as the middle two marker alleles were both the same as Sumai 3, it is possible that this genotype also contains *Fhb1*. The only genotype to contain the 5A QTL was Alsen and no genotypes except for Sumai 3 and China 2 contained the entire 6BS QTL (*Fhb2*).

The marker analyses revealed that China 1 gave a null allele result at all loci tested on chromosome 3BS. This suggested that this genotype may be missing a chromosomal segment at the distal end of the short arm of chromosome 3BS. To investigate this possibility, the composite wheat map from the KOMUGI database was used to identify markers at additional loci on this chromosome (http://www.shigen.nig.ac.jp/wheat/komugi/top/top/jsp). The markers *Xgwm389*, *Xwmc54*, *Xwmc78* and *Xwmc231* were chosen. In Figure 3.5 the relative position of these markers is given. PCR products were detected for all four markers (Figure 3.6).

Rht semi-dwarfing gene allele combinations were examined using the same genotypes. Genotypes can either have the *Rht-B1a* allele conferring a tall phenotype, or the *Rht-B1b* allele conferring a short phenotype. The same applied for the D genome alleles. In this analysis, tall alleles on the B genome were seen for China 2, China 3, Consort, CIMMYT 2, Sumai 3 and Ning 7840, and tall alleles on the D genome were seen for China 1, China 2, China 4, China 5, China 6, China 7, China 8, USA1, CIMMYT 1, Bobwhite, Alsen, Sumai 3, and Ning 7840 (Table 3.5).

Analysis of the genotypes using DArT markers was based on the presence or absence of over 1800 markers. Principal component analysis revealed clustering of the genotypes (Dr. Hai-Chun Jing, Rothamsted Research, pers. comm.), shown in Figure 3.7. This clustering analysis shows the close relationship between Sumai 3 and China 2, another Sumai 3, confirming the information given with this genotype. Wuhan and Gottingen 3 are unrelated to any of the other genotypes tested, and Petrus and Rialto group together as a pair. Two main clusters are evident consisting of Ning 7840, China 4, CIMMYT 1, and CIMMYT 2, and Alsen, China 1, China 3, China 5, China 6, and China 8.

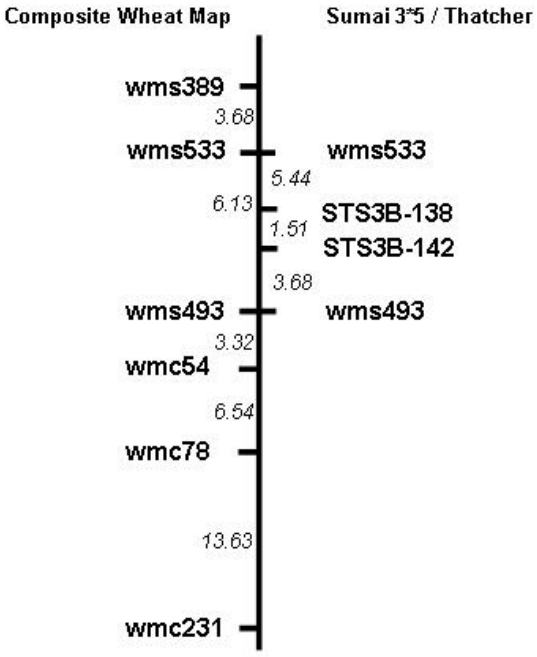


Figure 3.5 Schematic representation of the tip of the short arm of chromosome 3B. Map distances in cM (italics) are given from the two maps which make up this map. The composite wheat map from the KOMUGI database on the left, covers the region wms389 to wmc231 but does not include the markers STS3B-138 and STS3B-142. The second map, from the mapping population Sumai 3*5/Thatcher on the right covers the region wms533 to wms493 all inclusive (adapted from Cuthbert *et al*. (2006)).

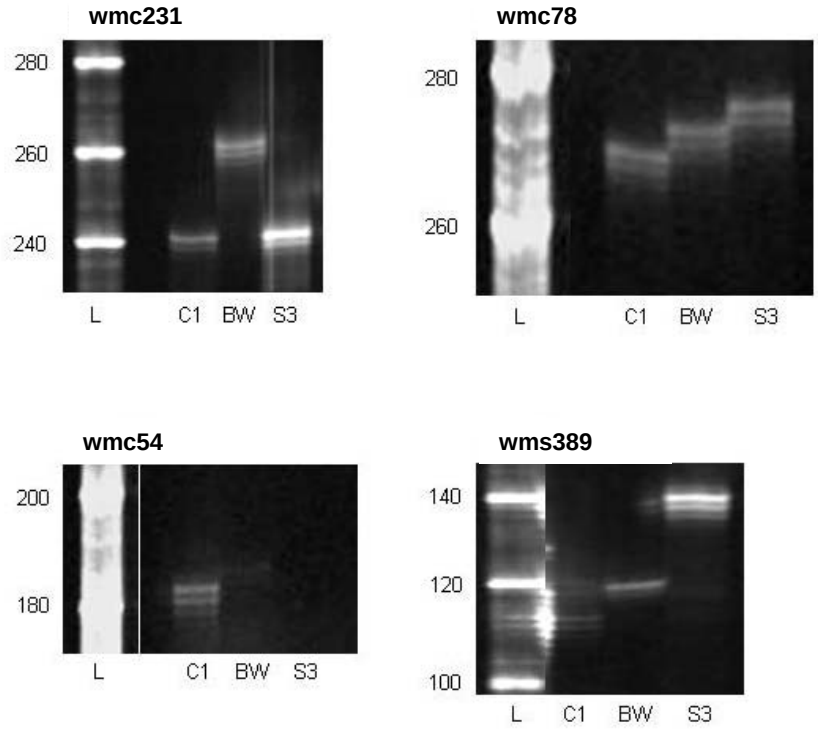


Figure 3.6 PCR products obtained for the markers wmc231, wmc78, wms389 and wmc54 on chromosome 3B from the genotypes China 1 (C1), Bobwhite (BW) and Sumai 3 (S3). Size ladders (L) are given in base pairs.

Table 3.5 *Rht* allele comparison in a selection of wheat genotypes used throughout this project

Genotype	B genome		D genome	
	Rht-B1a	***Rht-B1b***	***Rht-D1a***	***Rht-D1b***
China 1		✓	✓	
China 2	✓		✓	
China 3	✓			✓
China 4		✓	✓	
China 5		✓	✓	
China 6		✓	✓	
China 7		✓	✓	
China 8		✓	✓	
USA1		✓	✓	
Consort	✓			✓
CIMMYT 1		✓	✓	
CIMMYT 2	✓			✓
Bobwhite		✓	✓	
Alsen		✓	✓	
Sumai 3	✓		✓	
Ning 7840	✓		✓	

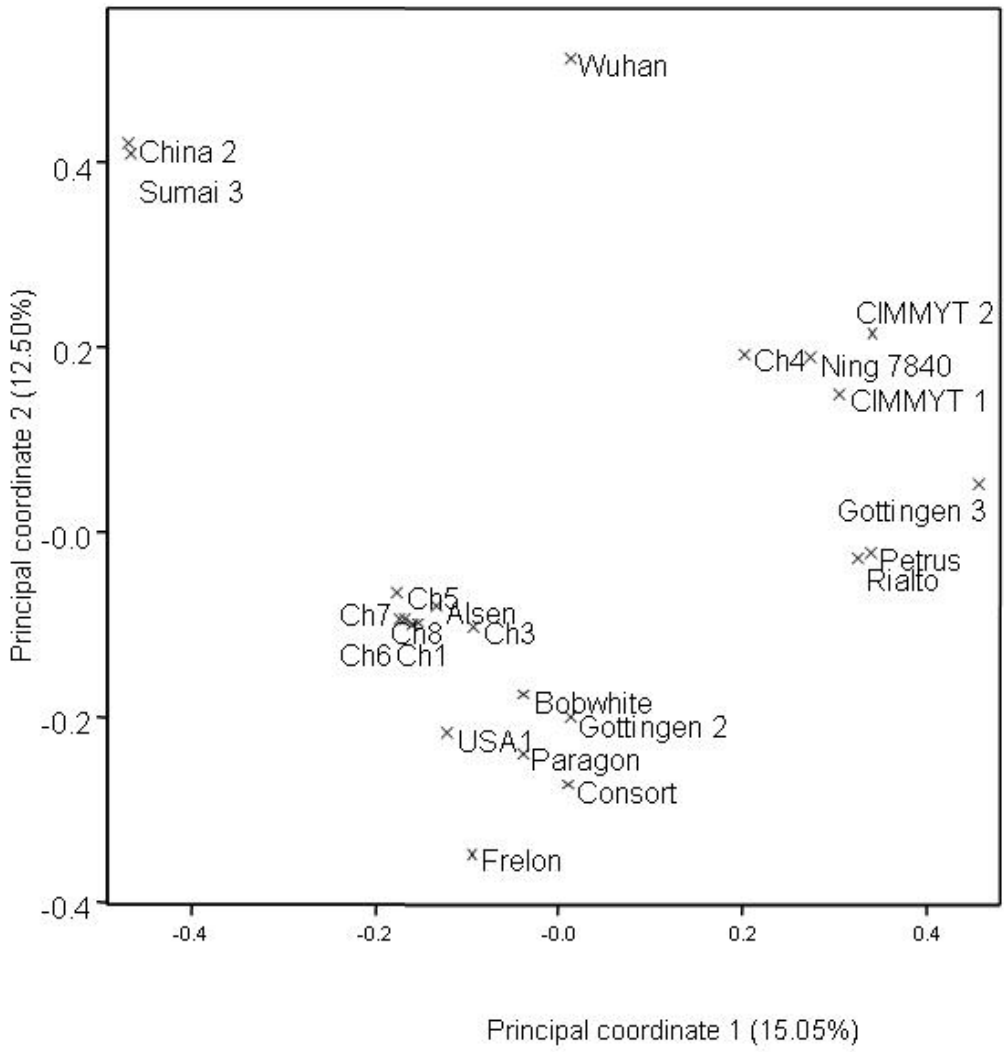


Figure 3.7 Principal coordinate analysis separating the wheat genotypes according to presence of DArT markers.

Forming a close group to the second cluster are Bobwhite, Gottingen 2, USA1, Paragon, Consort, and Frelon. The data from Bobwhite and Ning 7840 should be interpreted with caution however, as the positioning of these genotypes in this analysis is based on only 1000 markers compared to over 1800 for the other genotypes.

3.4 Discussion

The aim of these experiments was to confirm the uniqueness of the wheat genotypes used in field evaluations in this project (see Chapter 4). Recent advances in the mapping of resistance loci in Sumai 3 and other sources has allowed the haplotyping of resistant material to aid the pyramiding of resistance QTL (McCartney et al., 2004; Yu et al., 2006). In this study, the SSR and STS band haplotypes of 15 genotypes were compared to those obtained from Sumai 3. This method is a quick and easy way of differentiating between the genotypes at three loci and demonstrates that no genotype except for China 2 had all the tested QTL in common with Sumai 3.

Previous pedigree information from Dr. Binjie Gan (Appendix A1) suggested that only one of the Chinese genotypes was related to Sumai 3. China 2 was included as another source of Sumai 3 and on the basis of these results China 2 contains all three resistance QTL. None of the other Chinese genotypes contained the entire QTL at either 3BS or 6BS, thus confirming that at these loci, the genotypes are not related to Sumai 3. The other genotypes included in this study demonstrated a range of haplotypes and also confirmed the known pedigree of some of the genotypes. Ning 7840 had the same marker alleles as Sumai 3 at all four loci on chromosome 3B, which indicates the presence of *Fhb1*. Ning 7840 is reported to contain Sumai 3 in its pedigree (Bai et al., 1999). Alsen is also reported to contain Sumai 3 in its pedigree and had the same marker alleles as Sumai 3 at five of the seven loci, three of the four of *Fhb1*, one on one side of *Fhb2* and the marker wms304 for the QTL on chromosome 5, suggesting the presence of the 5AS QTL. For the remainder of the genotypes tested, the only complete QTL detected was on 3BS in CIMMYT 2. These results provide evidence that the genotypes going into the field trial

and glasshouse experiments are novel, and so any resistance observed would be useful in pyramiding resistance QTL or genes within a breeding programme. Interestingly, China 3 contains 2 of the flanking marker alleles for the 3BS QTL, but crucially, misses the allele at STS3B-142. Other genotypes, such as China 6, 7, and 8, USA1 and Consort contain only this allele, which might suggest that there is a high level of diversity between the markers wms493 and STS3B-142. This high level of recombination is well recognised on the wheat genomes, with higher recombination rates at the tips of chromosomes (Akhunov et al., 2003), thus highlighting the need for tightly linked markers when selecting for this QTL.

The recently published data regarding the importance of the *Rht* alleles on susceptibility to FEB lead to the additional marker analysis carried out in our group (Carlos Bayon, Rothamsted Research). Previous data suggested that type I resistance was reduced in the presence of *Rht-B1b* and *Rht-D1b* whilst type II resistance increased in the presence of *Rht-B1b* (Srinivasachary et al., 2009). The genotypes analysed here presented a mixed allele set with different combinations of the alleles on the two genomes. Further analysis of the resistance reactions for these genotypes in addition to the presence of these deleterious alleles will give further insight into the strength of the resistance response. For example, CIMMYT 2 has the *Rht-D1b* allele, which means that if this is a resistant genotype, the resistance mechanisms have to overcome the susceptibility associated with the genomic locus harbouring *Rht-D1b* as well as providing good resistance.

The haplotype for China 1 on chromosome 3BS showed null alleles at all four marker loci. This result suggested that the entire tip of the short arm of the chromosome may be absent in China 1. To explore this further, additional marker loci were examined in China 1, Bobwhite, and Sumai 3. PCR products were detected when primers are used for the four additional loci. This indicated that the short arm of 3BS is intact, but that at least 10 cM is highly dissimilar in China 1. This apparent deletion has been reported elsewhere for the FEB susceptible genotype Apogee (Mackintosh et al., 2006). Null alleles were seen for the same markers as those used in this study, and the authors suggested

that the 3BS QTL had been deleted, therefore leading to the susceptibility of the genotype.

This kind of haplotyping study only gives an indication of relatedness at three loci. It is possible that genotypes with all three QTL may not be resistant (Yu et al., 2006). For a more global analysis of relatedness across the genomes of these genotypes, DArT marker analysis was used. This demonstrated that the genotypes could be grouped into seven main clusters. Pedigree information for the Chinese genotypes (Appendix A1) can be linked into the results from this analysis. China 2 separates with Sumai 3, as expected. China 4 clustered with non-chinese genotypes, and this is reflected in the pedigree information which states that it does not have any parents in common with China 1 and China 6. A large cluster consisting of the remaining Chinese genotypes was also expected as China 1 and China 6 are related, and China 5, China 7, and China 8 are related. Interestingly, China 3 is not related to any of the other genotypes and also forms part of this cluster, along with Alsen. The inclusion of Alsen in this cluster is interesting, and suggests that there may be common parents at some point in the pedigree history between the Chinese genotypes and Alsen. It would be expected however that Sumai 3 derivatives Alsen and Ning 7840 should cluster closer to Sumai 3. The questionable reliability of the data for Ning 7840 could explain this anomalie, however the apparent distance between Sumai 3 and Alsen suggests that the Sumai 3 component of Alsen is relatively small. This contrasts with the SSR marker data which suggests that Alsen is similar to Sumai 3 at two out of the three loci tested. Such an outcome would be expected in a breeding programme, which used marker assisted introgression of the desired resistance QTLs followed by recurrent backcrossing to the susceptible elite parent to achieve the final commercial cultivar. A further point of interest is whether the clustering of the genotypes based on the DArT marker results is consistent with allele combinations for the SSR marker analysis, i.e. within each cluster, do all genotypes have the same allele at a particular SSR locus? For the cluster consisting of Sumai 3 and China 2, there is complete agreement of the alleles which, as discussed previously, is to be expected. For the other clusters, there

was no agreement between the allele combinations at any of the 3BS loci. At each of these loci, genotypes from the same cluster displayed alleles both the same and different to Sumai 3. Good agreement was seen for wms304 and wms644. This comparison is only of limited value however as the SSR results are merely presence or absence of an allele similar to Sumai 3 at three specific loci. A better comparison would be between specific allele sizes measured in base pairs and the DArT clustering, as allele sizes for the non-Sumai 3 alleles varied considerably. In addition, the DArT markers cover the entire genome and the SSR markers in this study cover only three loci, so the agreement between the two data sets would be improved when comparing the 3B, 5A, and 6B regions of the DArT data with the SSR marker data. But so far not all the wheat DArT markers have been successfully assigned chromosome locations. In summary, the genotypes tested here all show novel QTL combinations in comparison with Sumai 3, with no genotypes showing a complete set of all three QTL, except for the China 2.

CHAPTER 4: EVALUATION OF RESISTANCE UNDER UK FIELD CONDITIONS

4.1 Introduction

Currently, the best methods for selecting wheat genotypes conferring resistant to FEB involve the inoculation of adult plants with *Fusarium* conidia. There are two main approaches: firstly, plants can be examined under glasshouse conditions (for example Bai et al., 2001b; Jiang et al., 2006a) and secondly, under field conditions (for example Buerstmayr et al., 2003; Somers et al., 2003). Each method has its own advantages. Glasshouse conditions allow for a more controlled environment, thus eliminating variation due to the weather. In the UK, specialist glasshouse facilities also allow the use of quarantine *Fusarium* isolates, such as reporter strains (see Chapter 5). Field conditions on the other hand allow wheat plants to develop fully because of unlimited root growth, as opposed to the limited root development within a pot, which may play a part in the outcome of the experiment. However, field conditions are more variable both within and between seasons.

In this study, a series of consecutive field trials was chosen as the best method for studying the resistance of 24 wheat genotypes. The wheat genotypes selected for the field trial are given in Table 3.1, chapter 3 and include the genotypes used in the SSR and DArT marker analyses, along with some extra genotypes. Petrus and Frelon were included as the resistant benchmark cultivars from Germany and France, respectively. Petrus has been shown to exhibit stable resistance across environments and lower DON accumulation in grain (Gosman et al., 2007). Rialto and Paragon are UK bread wheat varieties, included as susceptible controls. Wuhan originates from China, and was previously shown to have high levels of resistance (Somers et al., 2003; McCartney et al., 2004). Gottingen 2 and Gottingen 3 are two breeding lines from Gottingen, Germany which were thought to have good resistance to FEB (C. Hilmar, Monsanto, Germany, unpublished data) and from the DArT analysis appears to have come from different genetic backgrounds (Figure 3.7).

Inoculation methods for field trials vary amongst researchers. Some opt for the spraying of *Fusarium* conidia onto wheat ears (for example Buerstmayr et al., 2003; Li et al., 2008), which allows large numbers of plants to be inoculated with ease. Point inoculation of ears, by injecting a conidia suspension (for example Schroeder and Christensen, 1963; Jiang et al., 2006a; Cuthbert et al., 2007; Li et al., 2008) directly into two to four spikelets per ear is an alternative method. Injection ensures all ears are inoculated, in comparison to spraying, which may allow some plants or ears to escape inoculation. A third method for inoculation is the spreading of *Fusarium* infected maize kernels or wheat grain, or infected maize stalks or wheat straw (for example Bateman et al., 2007). These are placed in the trial in the spring, and then the trial becomes inoculated over the subsequent months when the mature conidia or ascospores are dispersed by wind and rain splash or via artificial irrigation (see Chapter 1). This third method has the advantage of infecting a large area with relative ease, but again, individual plants may escape infection. The different resistance types can be evaluated using these methods, with type I and type II resistances being evaluated when using a spray or grain-spreading method. Point inoculation evaluates only type II resistance, as the conidia are delivered directly into the floret, thereby circumventing the initial penetration stage (Miedaner et al., 2003a; Bai and Shaner, 2004). For the field trials in this project, a double conidia spray method was used and the percentage of ears infected per plot were taken to represent type I resistance, the percentage of spikelets infected per ear represented type II resistance.

A further measure of disease assessment is also employed in field studies of FEB resistance. The area under disease progress curve (AUDPC) gives a quantitative measure for the progress of visible disease, using the previously mentioned parameters. The AUDPC measures the area under a graph of disease severity over time (Shaner and Finney, 1977), and the equation is given below (1):

$$\text{AUDPC} = \sum_{i=1}^{n} ((Y_{i+n1} + Y_i)/2)] (X_{i+n1} + X_i] \qquad (1)$$

where Y_i = diseases score at the *i*th observation, X_i = time (days) at the *i*th observation, and n = total number of observations.

A large AUDPC value can be indicative of a high amount of disease. This may be as a result of a high amount of disease, but only in the later stages of the assessment, or a large area may also be seen when the disease scores are only moderate, but continuously throughout the season.

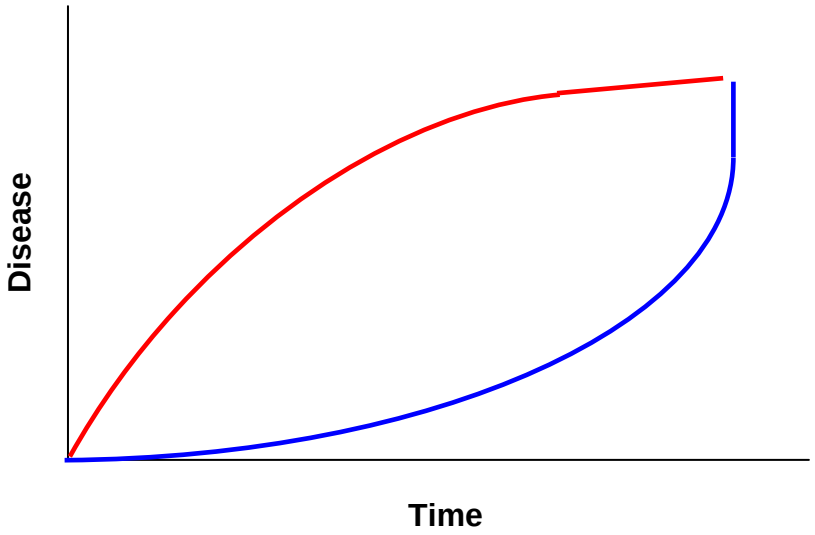


Figure 4.1 An example of two contrasting area under disease progress curves. Measurements show the progress of disease over time.

In Figure 4.1 the red line represents a high AUDPC because from the onset of infection there is a high rate of disease development. A curve of this shape suggests the genotype has low resistance, ears are infected early and then the infection progresses rapidly. The grain from this genotype is likely to contain large amounts of DON mycotoxin, unless other DON-related reduction mechanisms are also in operation. The blue curve on the other hand represents a more resistant genotype; early disease development is low, and then the rate of infection remains low, but at later time points disease development progresses more rapidly. This genotype would be expected to accumulate less DON mycotoxin, unless other DON-related accumulation mechanisms are also in operation. In the diagram both genotypes result in the same final level of disease. This highlights the importance of using many assessment dates in the field and then checking the AUDPC when two genotypes appear to have the same final levels of disease

resistance/susceptibility. To improve the accuracy of field assessments, often experimenters base the assessment dates on thermal time (Lovell et al., 2004). The use of thermal time ensures that the variation due to the weather conditions is limited by combining temperature data with number of days after inoculation. Therefore, if the weather conditions are warm, the disease is likely to progress quicker, and the disease should be assessed at shorter time intervals.

Previous field trials conducted by other research groups have surveyed a wide variety of wheat genotypes from sources around the world. The resistant genotypes identified so far have been discussed in Chapter 1. The Chinese genotypes in this UK study were tested for resistance against a number of known resistant controls, including Sumai 3, Ning 7840 and Wuhan.

4.2 Materials and Methods

4.2.1. Field trials

Twenty four hexaploid wheat genotypes (Tables 3.1 and 4.3) were evaluated for resistance in field trials at Rothamsted Research during the field seasons 2005/2006 and 2006/2007. In 2005/2006 genotypes were arranged in two blocks of 3 replicates. Replicates four, five and six, were sown approximately two weeks after replicates one, two and three to distribute flowering times. Flowering times did not differ greatly using this method, and in 2006/2007 genotypes were arranged in one block of six replicates. Within each replicate, the 24 genotypes were arranged according to a randomised complete block design. Field trial design plans are given in the appendix (Appendix A2 and Appendix A3). Each plot (0.9m x 0.54m) consisted of three rows of approximately 15 plants. In each plot, ten ears and one spare ear all at a similar developmental stage (30% anthesis) were tagged immediately before inoculation. All further disease assessments were made on these ten ears. Crop canopy moisture was monitored using a wet leaf moisture sensor (Access Irrigation Ltd., Northampton, UK). Once the sensor had dried adequately, the mist irrigation was switched on for 1.5 minutes. This occurred roughly every half hour, during the day from 0600 hrs until 2000 hrs. The mist irrigation system was used from the start of the inoculation period until the end of the

disease assessment schedule. The mist irrigation equipment was acquired from Access Irrigation Ltd., Northampton, UK.

The field plots were artificially inoculated with a water suspension of one naturally occurring UK isolate of *Fusarium culmorum* (UK-99), and two naturally occurring UK isolates of *Fusarium graminearum* (Fg576.1 and Fg602.10). These were kindly provided by G. Bateman, Rothamsted Research. A total of 30 ml of inoculum per plot was applied at a constant rate of 10 ml/second. The concentration of the inoculum was 6×10^3 spores/ml for UK-99 and 1.3×10^4 spores/ml for Fg576.1 and Fg602.10 in 2006. In 2007, all three isolates were applied at a concentration of 1.3×10^4 spores/ml. *F. culmorum* inoculum was prepared from concentrated samples of *Fusarium* conidia harvested from potato dextrose agar (PDA) plates and the *F. graminearum* inoculum was prepared from concentrated samples of *Fusarium* conidia harvested from mung bean media (Bai et al., 2000; Evans et al., 2000). Concentrated spores (1.3×10^6 conidia/ml) were stored as 10 ml ice cubes at -20ºC, and only defrosted and diluted to the concentration required in distilled water just prior to inoculation. The CP5 Maxipro sprayer (Cooper Pegler Sprayers, Green Tech Ltd., York, UK) was used for artificial inoculation. The sprayer device was fitted with a modified metallic hood containing two spray nozzles. This permitted the inoculum to be targeted to the three ear rows in each plot and to reduce the wind dispersal of inoculum. Plots were inoculated when the ears in the plot were at 30% anthesis, and then 1-2 days later at 50% anthesis. Inoculations were carried out in the evening to prevent the inoculum drying out in the warmer daytime. The irrigation was switched off on the afternoon prior to inoculation to allow plants to dry out, and was switched on again the following morning. A wind break 2m tall surrounded the trial to provide protection and help maintain a high humidity. Rainfall during inoculation is undesirable, and so plastic covers were produced to cover inoculated plots both before and after inoculation to allow the spores to adhere to the ear surface (Figure 4.2). These were placed over the inoculated plots for 6 hours prior to inoculation and remained on overnight depending on the forecast.

Figure 4.2 Covers for field trial plots in 2007. Exceptional rainfall increased the chance of inoculum being washed off after inoculation and so plastic covers were used to keep the plot dry immediately before and after inoculation. Also seen in this image is part of the 2 m tall wind break surrounding the entire trial.

Disease development was measured visually at day degree (DD) intervals based on thermal time (Lovell et al., 2004). The ten tagged ears were assessed by counting the number of bleached spikelets within each ear. The number of infected ears within the ten tagged ears was also counted. Assessments were carried out at twelve time points over each field season, at 30DD intervals. Data was analysed using GenStat v11 (Payne et al., 2005). Percentage disease data was transformed using the logit transformation (1)

$$\text{Logit}(P) = 0.5 \times \ln((P+0.5)/(100.5-P)) \quad (1)$$

where P is the percentage disease score

The transformation ensured equal variance in the data before further analysis with analysis of variance (ANOVA). Area under disease progress curves (AUDPC) were also calculated in GenStat using both the percentage of ears infected per plot and percentage of spikelets per ear infected until the time point DD550.

At the time of harvest, the plants were measured for their height and the distance between the bottom of the ear and the flag leaf (the peduncle length), to compare plant stature in the different seasons. From each plot, the fully mature tagged ears were harvested separately into brown paper bags and left in a polytunnel to continue drying before storage in cool, dark conditions. Ears were carefully threshed using a static thresher (Maschinenbau Ried Innkr, Vienna, Austria) and the seed stored at 15 ºC prior to further analysis. Hand threshing of samples was attempted in 2007 to limit the loss of shrivelled kernels, but this method was not suitable for processing a large number of samples.

4.2.2. Mycotoxin analysis by GC-MS

The moisture content of the grain samples was measured and adjusted to approximately 15% by adding a volume of water (ml) equal to the weight of the sample x percentage increase x 0.01. The grain was then milled using the Brabender Quadrumat Junior Mill, using clean grain harvested from glasshouse grown plants, provided by K. Plumb (Rothamsted Research), to clean the mill

between each sample. The bulked grain harvested from each plot was milled in a specific order, starting with plots which had recorded the lowest disease through to those which had the highest disease. The aim was to avoid cross contamination. The mycotoxin content was then measured using GC-MS analysis, based on a protocol devised by Tacke and Casper (1996). One sample was analysed from each plot, with no technical replicates. For each sample, 1.000 ± 0.001g sample was placed in a 2-dram tall form vial (Camlab). 3.0 ml 70:21 MeCN:H_2O and 1.0 ml of the internal standard solution (DOM-1, 2.5 ppm w/v in MeCN) (Biopure, Tulln, Austria) were added. The mixture was vortexed and left to extract at room temperature for 60 mins on an orbital shaker at 140rpm. Chromatography columns were prepared by mixing 50g C_{18} sorbent and 150g aluminium oxide and spreading in a thin layer in a crystallising dish which was left overnight exposed to the air. Solid phase extraction (SPE) barrels were fitted with a lower frit and the column was filled with 1.00 ± 0.01g of the sorbent, followed by another frit. The contents of the extraction vials were transferred to the columns and allowed to pass through. A 1.00 ml aliquot of the filtrate was evaporated to dryness in a 4 ml vial (Supelco). The residue was derivitised by adding 100 µl 100:1 trimethylsilyl-imidazole (TMSI): trimethyl-chlorosilane (TMCS) using a glass syringe. This was left to react at room temperature for 15 minutes, before 1 ml iso-octane was added to each sample followed by 1.0 ml distilled water was added. The vial was vortexed at full speed for 12 seconds and the upper organic layer was removed using a glass Pasteur pipette and transferred to a 2 ml autosampler vial prior to analysis using the Hewlett Packard MSD. In 2005/2006, GC-MS analysis was done on a Hewlett Packard 5890 Gas Chromatograph coupled to a 5970 MSD (Agilent Technologies, Santa Clara, CA). The column was a Zebron Guardian ZB-5 column with 5m integrated guard column (Phenomonex, Macclesfield, UK; 30 m x 0.25 mm internal diameter x 0.25 µm film). Five microlitre splitless injections were made at an inlet temperature of 300 °C. Helium was used as the carrier gas with a column head pressure of 105kPa. The oven was set to an initial temperature of 80 °C for 1 min, then ramped at 25 °C/min to 280 °C and held for 6 min. The GC interface was 250 °C and EI+ (electron ionisation) mass

spectra were acquired using selected ion monitoring (SIM) of m/z 193, 361, 391 (DOM-1), m/z 103, 235, 422 (DON), m/z 103, 277, 392 (3/15-ADON) and m/z 103, 191, 289 (NIV). Dwell times for all ions were 50 ms. Quantification of was based on peak areas for the mass chromatograms for ions m/z 361 (DOM-1) and m/z 103 (DON).

In 2006/2007, sample preparation was carried out using the same method as 2005/2006, but a new machine was used for the final GC-SM analysis. The analysis was done on a 7890 Gas Chromatograph coupled to a 5975C Inert MSD (Agilent Technologies, Santa Clara, CA). The column was a Zebron Guardian ZB-5 column with 5m integrated guard column (Phenomonex, Macclesfield, UK; 30 m x 0.25 mm internal diameter x 0.25 µm film). 2.5 microlitre splitless injections were made at an inlet temperature of 250 °C. Helium was used as the carrier gas at 2.0 ml/min, constant flow. The oven was set to an initial temperature of 70 °C for 2 min, then ramped at 17 °C/min to 350 °C and held for 1.5 min. The GC interface was 320 °C and EI+ (electron ionization) mass spectra were acquired using selected ion monitoring (SIM) of m/z 103, 181, 193, 391 (DOM-1), m/z 103, 193, 197, 235 (DON), m/z 103, 117, 163, 193 (3-ADON), m/z 103, 117, 193, 235 (15-ADON), and m/z 103, 191, 289 (NIV). Dwell times were 50ms for all ions except m/z 289 (NIV) which was 100ms. For all ions 50 ms Quantification of was based on peak areas for the mass chromatograms for ions m/z 361 (DOM-1) and m/z 103 (DON).

Standard curves were generated using following concentrations of trichothecene standards (DON, 3-ADON, 15-ADON, NIV, and DOM-1(Biopure, Tulln, Austria): 50ppb, 100 ppb, 250 ppb, 500ppb, 1 ppm, 2.5 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm and are given in the appendix (A4 and A5). Peak areas for the mycotoxins were calculated automatically using the quantify procedure within Masslynx (Micromass, Manchester, UK), following conversion of the data files from their native (HP) format to Masslynx files using the Mass Transit file conversion utility program (Palisade, Newfield, New York, USA). Data was analysed using GenStat v11 (Payne et al., 2005). DON mycotoxin data was transformed using the natural log transformation to ensure equal variance in the data before analysis using ANOVA. In 2007, a bulked analysis was also carried

out in parallel with the standard analysis detailed above, to try and reduced some of the variability seen in the method. Samples were generated using milled flour from all plots to produce a 1.000 ± 0.001g aliquot which was made up of all 6 biological replicates from the field (one sixth of a gram from each plot). This was repeated three times and the samples were analysed in the same way as the other 2007 samples.

4.3 Results

4.3.1. Preliminary data from 2004/2005

Preliminary data from a six replicated plot Rothamsted field trial in 2004/2005 suggested that five of the new Chinese genotypes tested showed comparable levels of resistance to Sumai 3 (Tables 4.1 and 4.2). Unfortunately due to space constraints, not all of the new Chinese material could be accommodated in this trial. Due to the nature of the data, statistical significance was determined on transformed data (as described in section 4.2). Mean untransformed data is also included for ease of data interpretation. At DD610, China 6 and China 4 were significantly more resistant than Sumai 3; China 3 and China 1 were of equal resistance, and China 7 was slightly less resistant when assessed as the percentage of ears infected per plot (Table 4.1). At the same time point, considering the percentage of spikelets infected per ear, China 6 was significantly more resistant than Sumai 3. China 4 and China 3 were equally resistant to Sumai 3, whilst China 1 and China 7 were slightly less resistant (Table 4.2). These promising results suggested further field testing was necessary and all genotypes were included in the 2005/2006 and 2006/2007 field trials.

4.3.2. Field trial establishment and implementation

All twenty four genotypes were evaluated for resistance to FEB in field trials over two years (Figure 4.3). To stagger genotype flowering over a period of a few days, the sowing was split into two in 2005. The sowing of 18 out of 24 wheat genotypes (Table 4.3) in replicates 1, 2 and 3 took place on 07/10/05, and in replicates 4, 5 and 6 on 20/10/05. The remaining six genotypes with a

Figure 4.3 The field trial at Rothamsted Research in 2006. Mist irrigation provided continued humidity to enhance disease progression.

Table 4.1 Percentage of ears infected per plot in the 2004/2005 field trials*

Code	Genotype	Ears DD610	Logit (Ears DD610)
27	**China 6**	1.00	-1.444
25	**China 4**	1.94	-1.395
12	Ning 7840	3.33	-1.322
24	**China 3**	4.27	-1.273
18	CIMMYT 1	5.00	-1.271
28	**China 1**	6.77	-1.171
19	CIMMYT 2	8.33	-1.119
11	Sumai 3	8.33	-1.071
16	Gottingen 2	13.33	-0.990
26	**China 7**	14.43	-0.785
13	Wuhan	15.00	-0.767
14	USA1	23.33	-0.562
17	Gottingen 3	26.67	-0.498
22	Paragon	75.00	0.515
23	Bobwhite	84.43	0.777
6	Consort	86.67	0.929
7	Rialto	90.00	1.032
15	Bobwhite	93.33	1.123
	SED		0.2669

Table 4.2 Percentage of spikelets infected per plot in the 2004/2005 field trials*

Code	Genotype	Spikelet DD610	Logit (Spikelet DD610)
27	**China 6**	-0.060	-2.812
18	CIMMYT 1	0.300	-2.693
12	Ning 7840	0.220	-2.686
25	**China 4**	0.290	-2.651
24	**China 3**	0.400	-2.607
16	Gottingen 2	0.740	-2.551
19	CIMMYT 2	0.540	-2.502
11	Sumai 3	0.530	-2.468
28	**China 1**	0.740	-2.456
13	Wuhan	0.950	-2.207
26	**China 7**	1.250	-2.147
17	Gottingen 3	1.850	-2.008
14	USA1	7.060	-1.309
22	Paragon	12.310	-0.982
15	Bobwhite	19.160	-0.718
23	Bobwhite	22.620	-0.571
7	Rialto	30.040	-0.426
6	Consort	40.380	-0.198
	SED		0.2191

Green shading indicates genotypes not significantly different to Sumai 3 (underlined). The Chinese genotypes are highlighted in bold.

* Daudi, 2007

spring habit (V18 CIMMYT1, V19 CIMMYT 2, V23 Bobwhite, V30 China 2, V31 China 5 and V32 China 8) were sown on 07/04/06 (all 6 replicates). This late sowing was actually a re-sowing following slug damage earlier in the season on all the early March sown plots. It should be noted that due to this late sowing, the plants for V18 CIMMYT 1 and V19 CIMMYT 2 were severely stunted at the time of anthesis this caused the data to be considered unreliable for the 2005/2006 field season. In the 2006/2007 field trial, the split sowing was removed and all six replicates from all 24 genotypes were sown on 05/10/06. Rodent damage in the 2006/2007 field season meant that the data for China 7 was incomplete, and as such has been removed from the analysis for this year. Occasional cases of ear damage caused some data points for the percentage of ears per plot or percentage spikelets per ear to decrease over time.

In 2006, the first inoculation occurred on 24/05/06 and the last inoculation occurred on 06/07/06 (Table 4.3). In 2007, the first inoculation occurred on 27/04/07 and the last inoculation occurred on 04/06/07 (Table 4.4). It is noticeable that the first date of inoculation in 2007 is approximately 1 month earlier than that of 2006. The two field seasons differed in weather conditions, and for this reason the average temperature and rainfall for each year are presented in Figures 4.4 and 4.5. The field season 2005/2006 was typically warm and dry, with some exceptionally hot days in July and early August. Rainfall was generally low, with an occasional wet day. Conversely, the field season 2006/2007, started with an exceptionally hot and dry April and May but thereafter was typically cooler and wetter, with consistent rain over the summer period.

To establish whether these conditions altered plant stature, plant height and peduncle length data for the tagged ears were compared and are presented in Tables 4.5, 4.6, 4.7 and 4.8. Mean values for the trials are similar across the two years, suggesting that the variation in weather between the years did not adversely affect the plant architecture in the trials as a whole. The protection of the field trial by the wind break and the irrigation of the trial from early anthesis onwards would have evened out rainfall differences, resulting in

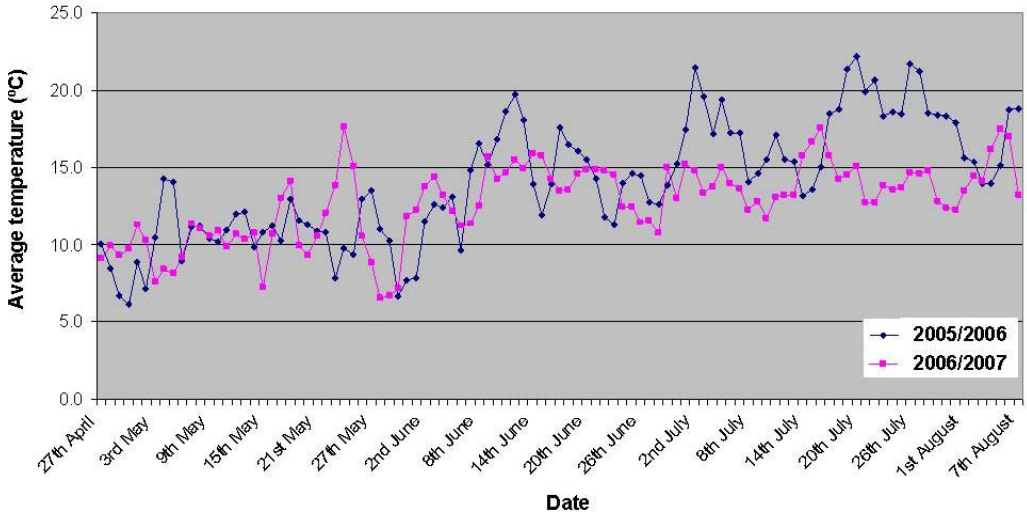


Figure 4.4 Average temperature over the field seasons 2005/2006 (blue line) and 2006/2007 (pink line). Source: Rothamsted Meteorological Station, Rothamsted Research.

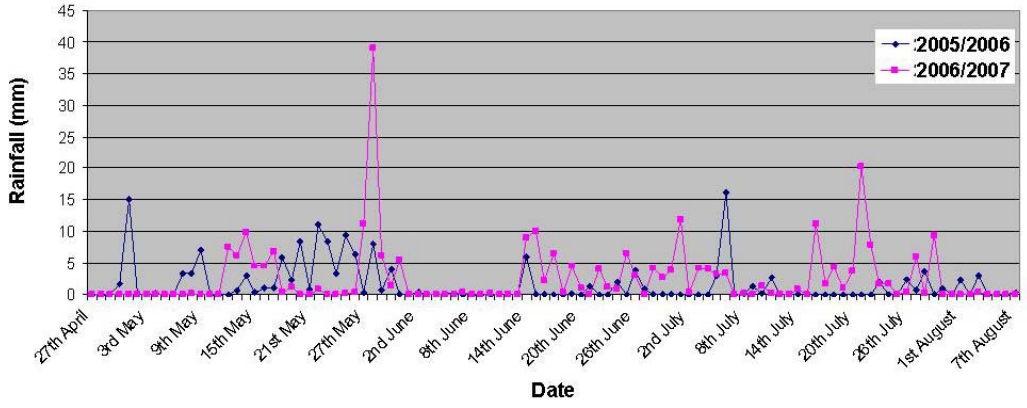


Figure 4.5 Rainfall over the field seasons 2005/2006 (blue line) and 2006/2007 (pink line). Source: Rothamsted Meteorological Station, Rothamsted Research.

Table 4.3 2005/2006 Inoculation dates (given in days after 24th May 2006)

Code	Genotype	Inoculation Date							
		Rep 1	Rep 2	Rep 3	Mean (n=3)	Rep 4	Rep 5	Rep 6	Mean (n = 3)
2	Petrus	19	19	21	19.67	29	30	27	28.67
6	Consort	23	23	25	23.67	30	30	29	29.67
7	Rialto	19	19	19	19.00	27	27	27	27.00
10	Frelon	15	15	15	15.00	21	19	19	19.67
11	Sumai 3	4	4	4	4.00	15	15	15	15.00
12	Ning 7840	10	11	12	11.00	18	18	19	18.33
13	Wuhan	11	11	15	12.33	19	18	21	19.33
14	USA1	4	4	10	6.00	15	16	15	15.33
15	Bobwhite	10	12	13	11.67	16	18	18	17.33
16	Gottingen 2	15	16	16	15.67	22	23	23	22.67
17	Gottingen 3	15	16	16	15.67	23	23	23	23.00
18	CIMMYT 1	43	43	43	43.00	43	43	43	43.00
19	CIMMYT 2	43	43	43	43.00	43	43	34	40.00
22	Paragon	18	19	18	18.33	27	25	25	25.67
23	Bobwhite[1]	36	32	32	33.33	36	32	39	35.67
24	**China 3**	2	2	2	2.00	9	10	12	10.33
25	**China 4**	2	0	2	1.33	12	12	12	12.00
26	**China 7**	4	4	10	6.00	13	13	13	13.00
27	**China 6**	4	7	4	5.00	13	13	13	13.00
28	**China 1**	2	2	4	2.67	15	15	13	14.33
29	Alsen	11	11	13	11.67	18	18	23	19.67
30	**China 2**	27	27	29	27.67	32	30	29	30.33
31	**China 5**	29	29	32	30.00	32	32	32	32.00
32	**China 8**	36	32	32	33.33	30	34	36	33.33
Mean		16.75	16.71	17.92	17.13	23.25	23.21	23.33	23.26

Note. CIMMYT 1 and CIMMYT 2 are omitted from analysis in this year (grey shading)

[1] Bobwhite spring sown

Table 4.4 2006/2007 Inoculation dates (given in days after 27th April 2007)

Code	Genotype	Inoculation Date						
		Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Mean (n=6)
2	Petrus	37	39	37	37	39	39	38.0
6	Consort	39	37	39	36	37	39	37.8
7	Rialto	36	36	36	36	36	36	36.0
10	Frelon	26	26	27	26	27	27	26.5
11	Sumai 3	7	12	7	7	7	12	8.7
12	Ning 7840	29	29	27	26	29	24	27.3
13	Wuhan	16	14	11	13	9	13	12.7
14	USA1	20	18	16	20	20	18	18.7
15	Bobwhite	29	26	27	14	16	24	22.7
16	Gottingen 2	37	36	36	36	34	37	36.0
17	Gottingen 3	29	34	34	27	27	34	30.8
18	CIMMYT 1	12	13	7	7	13	13	10.8
19	CIMMYT 2	18	14	13	12	13	13	13.8
22	Paragon	39	39	39	39	39	37	38.7
23	Bobwhite	15	27	22	26	22	26	23.0
24	**China 3**	3	1	5	2	1	1	2.2
25	**China 4**	4	4	14	11	4	5	7.0
26	China 7	6	2	4	3	4	4	3.8
27	**China 6**	8	16	14	12	7	7	10.7
28	**China 1**	12	11	7	11	5	7	8.8
29	Alsen	16	22	22	13	15	16	17.3
30	**China 2**	7	6	7	7	6	5	6.3
31	**China 5**	8	7	6	7	6	11	7.5
32	**China 8**	4	1	1	4	1	4	2.5
Mean		19.0	19.6	19.1	18.0	17.4	18.8	18.7

Note. China 7 data is omitted from analysis in this year (grey shading)

Table 4.5 Plant height data (2005/2006)

Code	Genotype	Plant Height (cm)	Rank Plant Height (cm)	*Rht* alleles*
31	**China 5**	47.89	1	*Rht-B1b / Rht-D1a*
23	Bobwhite[1]	54.53	2	*Rht-B1b / Rht-D1a*
32	**China 8**	58.74	3	*Rht-B1b / Rht-D1a*
24	**China 3**	63.83	4	*Rht-B1a / Rht-D1b*
19	CIMMYT 2	66.23	5	*Rht-B1a / Rht-D1b*
6	Consort	67.69	6	*Rht-B1a / Rht-D1b*
27	**China 6**	68.27	7	*Rht-B1b / Rht-D1a*
15	Bobwhite	71.11	8	*Rht-B1b / Rht-D1a*
30	**China 2**	71.26	9	*Rht-B1a / Rht-D1a*
25	**China 4**	72.09	10	*Rht-B1b / Rht-D1a*
26	**China 7**	75.27	11	*Rht-B1b / Rht-D1a*
28	**China 1**	77.41	12	*Rht-B1b / Rht-D1a*
18	CIMMYT 1	77.90	13	*Rht-B1b / Rht-D1a*
13	Wuhan	79.76	14	*Rht-B1a / Rht-D1a*
29	Alsen	80.68	15	*Rht-B1b / Rht-D1a*
10	Frelon	81.31	16	*Rht-B1b / Rht-D1a*
12	Ning 7840	82.88	17	*Rht-B1a / Rht-D1a*
14	USA1	83.64	18	*Rht-B1b / Rht-D1a*
7	Rialto	86.39	19	*Rht-B1a / Rht-D1b*
11	Sumai 3	89.82	20	*Rht-B1a / Rht-D1a*
22	Paragon	91.85	21	*Rht-B1a / Rht-D1a*
2	Petrus	104.30	22	*Rht-B1a / Rht-D1a*
17	Gottingen 3	106.42	23	*Rht-B1a / Rht-D1a*
16	Gottingen 2	122.66	24	*Rht-B1a / Rht-D1a*
	Mean	78.41		
	SED	3.612		

Table 4.6 Peduncle length data (2005/2006)

Code	Genotype	Peduncle Length (cm)	Rank Peduncle Length (cm)	*Rht* alleles*
19	CIMMYT 2	4.21	1	*Rht-B1a / Rht-D1b*
18	CIMMYT 1	5.16	2	*Rht-B1b / Rht-D1a*
7	Rialto	5.53	3	*Rht-B1a / Rht-D1b*
6	Consort	6.79	4	*Rht-B1a / Rht-D1b*
23	Bobwhite[1]	8.12	5	*Rht-B1b / Rht-D1a*
31	**China 5**	8.16	6	*Rht-B1b / Rht-D1a*
32	**China 8**	8.56	7	*Rht-B1b / Rht-D1a*
10	Frelon	9.43	8	*Rht-B1b / Rht-D1a*
25	**China 4**	11.4	9	*Rht-B1b / Rht-D1a*
27	**China 6**	12.11	10	*Rht-B1b / Rht-D1a*
28	**China 1**	12.19	11	*Rht-B1b / Rht-D1a*
24	**China 3**	12.77	12	*Rht-B1a / Rht-D1b*
22	Paragon	12.82	13	*Rht-B1a / Rht-D1a*
15	Bobwhite	13.39	14	*Rht-B1b / Rht-D1a*
2	Petrus	15.58	15	*Rht-B1a / Rht-D1a*
17	Gottingen 3	16.38	16	*Rht-B1a / Rht-D1a*
14	USA1	17.35	17	*Rht-B1b / Rht-D1a*
30	**China 2**	17.73	18	*Rht-B1a / Rht-D1a*
26	**China 7**	18.18	19	*Rht-B1b / Rht-D1a*
13	Wuhan	18.89	20	*Rht-B1a / Rht-D1a*
12	Ning 7840	19.52	21	*Rht-B1a / Rht-D1a*
29	Alsen	22.88	22	*Rht-B1b / Rht-D1a*
16	Gottingen 2	23.68	23	*Rht-B1a / Rht-D1a*
11	Sumai 3	23.77	24	*Rht-B1a / Rht-D1a*
	Mean	13.53		
	SED	1.822		

* *Rht* allele data from chapter 3. Colour coding highlights the different allele combinations.

Table 4.7 Plant height data (2006/2007)

Code	Genotype	Plant Height (cm)	Rank Plant Height (cm)	*Rht* alleles*
24	**China 3**	58.72	1	*Rht-B1a / Rht-D1b*
32	**China 8**	58.96	2	*Rht-B1b / Rht-D1a*
31	**China 5**	60.19	3	*Rht-B1b / Rht-D1a*
25	**China 4**	62.73	4	*Rht-B1b / Rht-D1a*
27	**China 6**	64.69	5	*Rht-B1b / Rht-D1a*
26	China 7	65.35	6	*Rht-B1b / Rht-D1a*
19	CIMMYT 2	70.06	7	*Rht-B1a / Rht-D1b*
28	**China 1**	70.10	8	*Rht-B1b / Rht-D1a*
15	Bobwhite	71.14	9	*Rht-B1b / Rht-D1a*
29	Alsen	72.30	10	*Rht-B1b / Rht-D1a*
23	Bobwhite	73.51	11	*Rht-B1b / Rht-D1a*
13	Wuhan	75.29	12	*Rht-B1a / Rht-D1a*
6	Consort	75.33	13	*Rht-B1a / Rht-D1b*
30	**China 2**	76.80	14	*Rht-B1a / Rht-D1a*
18	CIMMYT 1	78.44	15	*Rht-B1b / Rht-D1a*
10	Frelon	82.41	16	*Rht-B1b / Rht-D1a*
7	Rialto	84.08	17	*Rht-B1a / Rht-D1b*
14	USA1	84.65	18	*Rht-B1b / Rht-D1a*
11	Sumai 3	86.36	19	*Rht-B1a / Rht-D1a*
22	Paragon	89.96	20	*Rht-B1a / Rht-D1a*
2	Petrus	90.54	21	*Rht-B1a / Rht-D1a*
17	Gottingen 3	96.91	22	*Rht-B1a / Rht-D1a*
12	Ning 7840	98.18	23	*Rht-B1a / Rht-D1a*
16	Gottingen 2	108.82	24	*Rht-B1a / Rht-D1a*
	Mean	77.31		
	SED	2.896		

Table 4.8 Peduncle length data (2006/2007)

Code	Genotype	Peduncle Length (cm)	Rank Peduncle Length (cm)	*Rht* alleles*
7	Rialto	6.00	1	*Rht-B1a / Rht-D1b*
2	Petrus	7.70	2	*Rht-B1a / Rht-D1a*
22	Paragon	7.79	3	*Rht-B1a / Rht-D1a*
6	Consort	8.97	4	*Rht-B1a / Rht-D1b*
17	Gottingen 3	10.02	5	*Rht-B1a / Rht-D1a*
31	**China 5**	11.20	6	*Rht-B1b / Rht-D1a*
10	Frelon	11.27	7	*Rht-B1b / Rht-D1a*
25	**China 4**	11.79	8	*Rht-B1b / Rht-D1a*
27	**China 6**	12.10	9	*Rht-B1b / Rht-D1a*
19	CIMMYT 2	12.39	10	*Rht-B1a / Rht-D1b*
24	**China 3**	14.78	11	*Rht-B1a / Rht-D1b*
28	**China 1**	15.19	12	*Rht-B1b / Rht-D1a*
16	Gottingen 2	15.45	13	*Rht-B1a / Rht-D1a*
15	Bobwhite	15.59	14	*Rht-B1b / Rht-D1a*
23	Bobwhite	16.70	15	*Rht-B1b / Rht-D1a*
14	USA1	18.80	16	*Rht-B1b / Rht-D1a*
32	**China 8**	19.34	17	*RhtB1b / Rht-D1a*
12	Ning 7840	19.53	18	*Rht-B1a / Rht-D1a*
26	China 7	21.02	19	*Rht-B1b / Rht-D1a*
13	Wuhan	21.91	20	*Rht-B1a / Rht-D1a*
29	Alsen	23.55	21	*Rht-B1b / Rht-D1a*
30	**China 2**	26.88	22	*Rht-B1a / Rht-D1a*
18	CIMMYT 1	27.02	23	*Rht-B1b / Rht-D1a*
11	Sumai 3	27.82	24	*Rht-B1a / Rht-D1a*
	Mean	15.95		
	SED	1.299		

* *Rht* allele data from chapter 3. Colour coding highlights the different allele combinations.

the principal difference being temperature. This difference could be compensated for by the choice of fungal isolates used for inoculation. As discussed in chapter 1, *F. culmorum* is prevalent in cooler climates, whereas *F. graminearum* is more prevalent in warmer climates. By using a mixed inoculum, infection would occur regardless of a small temperature difference. For some plots the selection of a single inoculation timing was difficult to ensure that there were 10 ears of the same growth stage. Therefore, the early flowering ears were tagged and sprayed twice as necessary. When further ears in the plot came into flower, they were tagged to make the numbers up to 11 (10 ears plus 1 spare in case of damage) and then sprayed twice as appropriate. As a consequence, some plots as a whole were sprayed 3 or 4 times with inoculum. Alternative spray equipment was used in 2006 due to damage to the original sprayer. The replacement sprayer was a hand held sprayer (Hozelock, Birmingham, UK). Inspection of the raw data indicated that both these modifications did not appear to generate any anomalies in the results.

When the height data is compared to the *Rht* allele data (see Chapter 3, Tables 4.5, 4.6, 4.7, and 4.8), a general trend can be seen, with genotypes carrying the allele combination *Rht-B1b* and *Rht-D1a* being generally shorter, and the allele combination *Rht-B1a* and *Rht-D1a* being generally taller. This was seen in both years. The allele combination *Rht-B1a* and *Rht-D1b* appeared to have a smaller effect on the height of the plants, with tall and short plants found carrying this allele combination. This trend was less apparent when considering the peduncle length.

4.3.3. Field evaluation of disease levels 2005/2006 and 2006/2007

Two types of disease assessment were carried out in the field: the percentage of spikelets per ear displaying symptoms (referred to as percentage spikelets) and the percentage of ears per plot showing symptoms (referred to as percentage ears). The time points DD310 and DD490 for 2005/2006 data and DD280 and DD520 for 2006/2007 data were chosen for in depth analysis, marking the start and end points of field assessments. There were some missing values in the raw data sets caused by occasional ear loss. The

percentage data were transformed before the analysis of variance was carried out to ensure equal variance in the data set, and it is from this transformed data set that statistical comparisons will be made. For the ease of comparison however, the untransformed means are included to indicate disease levels seen in the field. The standard error of the difference (SED) values reveal statistically significant differences between the genotypes across both years for both disease parameters measured. Since one of the aims of this project was to identify novel sources of resistance, disease scores were compared to the well known resistance source Sumai 3 using the SED values.

Type I resistance: Percentage of ears infected

The 2005/2006 and 2006/2007 field seasons were both highly conducive to *Fusarium* infection and good discrimination between genotypes was possible. In the 2005/2006 field season, the percentage of infected ears ranged from 11.5 - 98.1% (Tables 4.9 and 4.10). At both the early time point of DD310 and the late time point of DD490, Sumai 3 shows the highest levels of resistance, along with Ning 7840 and Wuhan. China 2 was also similar to Sumai 3 at DD310. These two results were to be expected because Ning 7840 contains Sumai 3 in the pedigree and China 2 is another Sumai 3 source from China. Wuhan, although not related to Sumai 3, is also well known as a source of resistance (see section 4.1). The other Chinese genotypes were significantly less resistant than Sumai 3, but still maintained moderate to high levels of resistance ranging from 40.0% of ears infected in China 1 to 88.7% of ears infected in China 3 at DD490. The other genotypes of interest which, although significantly different to Sumai 3, displayed moderate levels of resistance, included Gottingen 2 and USA1. In 2006/2007 the percentage of infected ears ranged from 7.5% to 100% (Tables 4.11 and 4.12). At DD280, Sumai 3 was once again the most resistant genotype, along with USA1, Frelon, Ning 7840, CIMMYT 2, Alsen, Gottingen 3, China 2, Wuhan, China 8 and China 1. At the later time point of DD520, USA1 demonstrated significantly more resistance than Sumai 3. CIMMYT 2, China 8, China 2, China 1, and Wuhan were not

Table 4.9 Percentage of ears infected at DD310 (2005/2006)

Code	Genotype	Ears DD310	Logit (Ears DD310)	Rank Logit (Ears DD310)
11	Sumai 3	0.0	-1.515	1
12	Ning 7840	1.7	-1.414	2
13	Wuhan	5.4	-1.260	3
30	**China 2**	4.4	-1.259	4
16	Gottingen 2	9.5	-1.068	5
14	USA1	12.9	-0.980	6
29	Alsen	12.9	-0.937	7
10	Frelon	15.0	-0.869	8
26	**China 7**	16.7	-0.796	9
25	**China 4**	14.1	-0.786	10
32	**China 8**	20.2	-0.679	11
28	**China 1**	21.7	-0.606	12
17	Gottingen 3	31.5	-0.480	13
19	CIMMYT 2	30.8	-0.391	14
2	Petrus	31.9	-0.331	15
27	**China 6**	37.1	-0.282	16
31	**China 5**	40.2	-0.268	17
15	Bobwhite	38.3	-0.226	18
22	Paragon	46.9	-0.142	19
23	Bobwhite[1]	49.1	0.000	20
24	**China 3**	53.8	0.167	21
18	CIMMYT 1	55.0	0.190	22
7	Rialto	60.0	0.380	23
6	Consort	70.5	0.484	24
	SED		0.3999	

Table 4.10 Percentage of ears infected at DD490 (2005/2006)

Code	Genotype	Ears (DD490)	Logit (Ears DD490)	Rank Logit (Ears DD490)
11	Sumai 3	11.5	-0.979	1
13	Wuhan	15.5	-0.912	2
12	Ning 7840	17.6	-0.862	3
30	**China 2**	24.3	-0.602	4
16	Gottingen 2	33.7	-0.400	5
28	**China 1**	40.0	-0.191	6
25	**China 4**	54.8	0.114	7
26	**China 7**	57.1	0.165	8
14	USA1	58.3	0.205	9
29	Alsen	66.0	0.318	10
17	Gottingen 3	66.5	0.414	11
10	Frelon	64.6	0.474	12
32	**China 8**	79.9	0.653	13
27	**China 6**	81.7	0.761	14
19	CIMMYT 2	83.3	0.840	15
22	Paragon	81.7	0.894	16
31	**China 5**	87.3	0.921	17
24	**China 3**	88.7	0.964	18
7	Rialto	86.3	0.969	19
15	Bobwhite	90.5	1.020	20
2	Petrus	86.7	1.038	21
18	CIMMYT 1	88.9	1.106	22
23	Bobwhite[1]	98.1	1.342	23
6	Consort	97.9	1.359	24
	SED		0.3955	

Table 4.11 Percentage of ears infected at DD280 (2006/2007)

Code	Genotype	Ears DD280	Logit (Ears DD280)	Rank Logit (Ears DD280)
11	Sumai 3	0.0	-1.520	1
14	USA1	1.5	-1.469	2
10	Frelon	1.5	-1.461	3
19	CIMMYT 2	1.5	-1.453	4
29	Alsen	1.5	-1.453	5
12	Ning 7840	1.7	-1.453	6
17	Gottingen 3	1.5	-1.446	7
30	**China 2**	1.7	-1.445	8
13	Wuhan	1.5	-1.414	9
32	**China 8**	4.5	-1.380	10
28	**China 1**	3.7	-1.292	11
23	Bobwhite	6.1	-1.253	12
27	**China 6**	6.1	-1.171	13
25	**China 4**	9.4	-1.164	14
16	Gottingen 2	10.6	-1.025	15
15	Bobwhite	16.1	-0.874	16
26	China 7	14.7	-0.872	17
24	**China 3**	16.7	-0.806	18
31	**China 5**	16.2	-0.765	19
18	CIMMYT 1	17.6	-0.765	20
2	Petrus	24.2	-0.708	21
22	Paragon	42.4	-0.157	22
6	Consort	43.9	-0.125	23
7	Rialto	50.0	-0.040	24
	SED		0.2355	

Table 4.12 Percentage of ears infected at DD520 (2006/2007)

Code	Genotype	Ears DD520	Logit (Ears DD520)	Rank Logit (Ears DD520)
14	USA1	7.5	-0.977	1
19	CIMMYT 2	14.4	-0.768	2
32	**China 8**	20.7	-0.648	3
11	Sumai 3	14.9	-0.621	4
30	**China 2**	23.5	-0.599	5
28	**China 1**	26.2	-0.578	6
13	Wuhan	27.6	-0.514	7
26	China 7	18.0	-0.488	8
29	Alsen	35.3	-0.301	9
25	**China 4**	51.9	0.046	10
27	**China 6**	54.5	0.074	11
24	**China 3**	57.8	0.145	12
31	**China 5**	61.5	0.199	13
18	CIMMYT 1	62.0	0.242	14
17	Gottingen 3	67.7	0.441	15
12	Ning 7840	75.9	0.651	16
23	Bobwhite	85.7	0.925	17
15	Bobwhite	91.5	1.164	18
16	Gottingen 2	93.0	1.215	19
10	Frelon	94.9	1.242	20
2	Petrus	100.0	1.535	21
6	Consort	100.0	1.552	22
22	Paragon	100.0	1.560	23
7	Rialto	100.0	1.568	24
	SED		0.2615	

significantly different from Sumai 3, whilst China 6 and China 4 showed moderate levels of resistance at both time points.

Type II resistance: percentage of spikelets infected

In 2005/2006, the percentage of spikelets infected ranged from 0.80 - 50.20%, with Ning 7840, Gottingen 2, Wuhan and China 2 all demonstratinglevels of resistance not significantly different to Sumai 3 at DD310 (Table 4.13). Later at DD490 the same genotypes, bar Gottingen 2 and China 2, were significantly similar to Sumai 3 (Table 4.14). China 1, China 4 and China 7, whilst significantly less resistant than Sumai 3, demonstrate high levels of resistance, with percentage scores of 3.9 - 10.15%. Moderate levels of resistance were seen in China 6, Alsen and USA1 at the final time point, and the remaining Chinese genotypes China 8, China 3, and China 5 showed medium levels of resistance. Predicted susceptible genotypes had between 31.70 and 50.20% infected spikelets. Results from the 2006/2007 field season showed that at DD280, Ning 7840, Gottingen 3, CIMMYT 2, Wuhan, Frelon, China 2, USA1, Alsen, and China 8 were all significantly similar to Sumai 3 (Table 4.15). China 1 was marginally more susceptible, and China 4, China 5 and China 6 more susceptible again. China 3 was the most susceptible. At the later time point of DD520, disease scores ranged from 0.80 – 79.60%, with USA1, CIMMYT 2, Wuhan, China 2, China 8 and China 1 not significantly different to Sumai 3 (Table 4.16). The remaining Chinese genotypes all demonstrated low levels of FEB infection, but were significantly more diseased that Sumai 3. Susceptible control genotypes demonstrated good levels of disease ranging from 43.80 – 79.60%.

4.3.4. Area under disease progress curve (AUDPC)

The area under disease progress curve allows the comparison of disease progression in the different genotypes. In 2005/2006, the AUDPC values for the percentage ears revealed large and statistically significant differences between resistant and susceptible genotypes, ranging from 1868 in Sumai 3 to 32027 in Consort (Table 4.17). Ning 7840, Wuhan, China 2 and

Table 4.13 Percentage of spikelets infected at DD310 (2005/2006)

Code	Genotype	Spikelet DD310	Logit (Spikelet DD310)	Rank Logit (Spikelet DD310)
11	Sumai 3	-0.05	-2.883	1
12	Ning 7840	0.71	-2.705	2
16	Gottingen 2	0.46	-2.655	3
13	Wuhan	1.05	-2.598	4
30	**China 2**	0.33	-2.551	5
29	Alsen	0.75	-2.400	6
14	USA1	1.00	-2.380	7
10	Frelon	0.95	-2.315	8
32	**China 8**	1.17	-2.203	9
25	**China 4**	0.94	-2.196	10
17	Gottingen 3	2.35	-2.141	11
28	**China 1**	1.53	-2.122	12
26	**China 7**	1.63	-2.116	13
2	Petrus	2.66	-2.080	14
22	Paragon	4.47	-1.955	15
19	CIMMYT 2	2.53	-1.823	16
27	**China 6**	3.24	-1.751	17
15	Bobwhite	2.94	-1.749	18
23	Bobwhite[1]	3.63	-1.632	19
31	**China 5**	5.54	-1.568	20
7	Rialto	6.17	-1.525	21
18	CIMMYT 1	5.11	-1.507	22
24	**China 3**	6.80	-1.390	23
6	Consort	8.74	-1.216	24
	SED		0.3540	

Table 4.14 Percentage of spikelets infected at DD490 (2005/2006)

Code	Genotype	Spikelets (DD490)	Logit (Spikelet DD490)	Rank Logit (Spikelet DD490)
11	Sumai 3	0.80	-2.351	1
13	Wuhan	4.14	-2.174	2
12	Ning 7840	2.45	-2.096	3
30	**China 2**	2.51	-1.865	4
16	Gottingen 2	5.50	-1.845	5
28	**China 1**	3.90	-1.687	6
25	**China 4**	6.43	-1.441	7
14	USA1	12.01	-1.324	8
29	Alsen	7.04	-1.306	9
26	**China 7**	10.15	-1.237	10
17	Gottingen 3	14.55	-1.011	11
10	Frelon	15.74	-0.985	12
2	Petrus	17.01	-0.976	13
27	**China 6**	17.36	-0.884	14
19	CIMMYT 2	21.15	-0.733	15
32	**China 8**	20.60	-0.692	16
18	CIMMYT 1	24.28	-0.654	17
24	**China 3**	26.70	-0.553	18
22	Paragon	31.70	-0.509	19
15	Bobwhite	29.75	-0.442	20
31	**China 5**	31.07	-0.429	21
7	Rialto	35.17	-0.388	22
23	Bobwhite[1]	45.66	-0.076	23
6	Consort	50.20	0.012	24
	SED		0.3966	

Table 4.15 Percentage of spikelets infected at DD280 (2006/2007)

Code	Genotype	Spikelet DD280	Logit (Spikelet DD280)	Rank Logit (Spikelet DD280)
12	Ning 7840	0.06	-3.039	1
17	Gottingen 3	0.12	-2.980	2
19	CIMMYT 2	0.07	-2.948	3
11	Sumai 3	0.00	-2.944	4
13	Wuhan	0.15	-2.892	5
10	Frelon	0.08	-2.879	6
30	**China 2**	0.10	-2.871	7
14	USA1	0.10	-2.821	8
29	Alsen	0.10	-2.809	9
32	**China 8**	0.29	-2.774	10
28	**China 1**	0.22	-2.716	11
23	Bobwhite	0.38	-2.703	12
25	**China 4**	0.65	-2.582	13
27	**China 6**	0.45	-2.543	14
16	Gottingen 2	0.72	-2.539	15
2	Petrus	1.93	-2.315	16
26	China 7	0.97	-2.312	17
31	**China 5**	0.92	-2.306	18
15	Bobwhite	1.41	-2.304	19
18	CIMMYT 1	1.26	-2.166	20
24	**China 3**	1.50	-2.134	21
22	Paragon	2.82	-1.797	22
7	Rialto	3.72	-1.704	23
6	Consort	4.24	-1.664	24
	SED		0.2270	

Table 4.16 Percentage of spikelets infected at DD520 (2006/2007)

Code	Genotype	Spikelet DD520	Logit (Spikelet DD520)	Rank Logit (Spikelet DD520)
14	USA1	0.90	-2.292	1
19	CIMMYT 2	2.00	-2.212	2
13	Wuhan	1.60	-2.174	3
30	**China 2**	1.40	-2.104	4
11	Sumai 3	0.80	-2.075	5
32	**China 8**	1.60	-2.054	6
28	**China 1**	3.00	-2.016	7
26	China 7	1.40	-1.840	8
29	Alsen	3.80	-1.657	9
27	**China 6**	4.80	-1.518	10
25	**China 4**	5.20	-1.505	11
12	Ning 7840	6.80	-1.410	12
18	CIMMYT 1	5.20	-1.407	13
17	Gottingen 3	7.50	-1.400	14
24	**China 3**	5.50	-1.394	15
31	**China 5**	6.70	-1.350	16
16	Gottingen 2	39.40	-0.241	17
15	Bobwhite	43.80	-0.142	18
23	Bobwhite	43.90	-0.122	19
10	Frelon	44.80	-0.115	20
2	Petrus	61.40	0.612	21
6	Consort	79.60	0.761	22
7	Rialto	73.90	0.950	23
22	Paragon	77.70	1.013	24
	SED		0.3390	

Table 4.17 AUDPC Ears (2005/2006)

Code	Genotype	AUDPC (Ears)	Rank AUDPC (Ears)
11	Sumai 3	1868	1
12	Ning 7840	3279	2
13	Wuhan	3411	3
30	**China 2**	4066	4
16	Gottingen 2	4765	5
28	**China 1**	7578	6
25	**China 4**	8515	7
17	Gottingen 3	9630	8
14	USA1	10230	9
26	**China 7**	10406	10
10	Frelon	11071	11
29	Alsen	11832	12
32	**China 8**	15607	13
2	Petrus	16289	14
19	CIMMYT 2	17094	15
27	**China 6**	17854	16
31	**China 5**	19048	17
15	Bobwhite	20092	18
18	CIMMYT 1	20243	19
22	Paragon	21006	20
23	Bobwhite[1]	22284	21
7	Rialto	24626	22
24	**China 3**	25178	23
6	Consort	32027	24
	SED	3457.6	

Table 4.18 AUDPC Spikelets (2005/2006)

Code	Genotype	AUDPC (Spikelet)	Rank AUDPC (Spikelet)
11	Sumai 3	138	1
30	**China 2**	397	2
13	Wuhan	442	3
16	Gottingen 2	447	4
12	Ning 7840	519	5
28	**China 1**	551	6
25	**China 4**	947	7
17	Gottingen 3	1230	8
26	**China 7**	1298	9
29	Alsen	1362	10
14	USA1	1707	11
10	Frelon	2162	12
27	**China 6**	2332	13
2	Petrus	2725	14
19	CIMMYT 2	3068	15
32	**China 8**	3163	16
18	CIMMYT 1	3730	17
22	Paragon	4533	18
15	Bobwhite	4961	19
7	Rialto	5366	20
31	**China 5**	5690	21
24	**China 3**	5730	22
23	Bobwhite[1]	6625	23
6	Consort	10369	24
	SED	1189.4	

Gottingen 2 all had values not significantly different to Sumai 3. China 1 and China 4 remained towards the more resistant end of Table 4.17; China 7 and China 8 demonstrating moderate values and China 6, China 5 and China 3 all had values towards the more susceptible end. The AUDPC values for the percentage spikelets showed similar differences with values ranging from 138 in Sumai 3 to 10369 in Consort (Table 4.18). No significant difference was found between Sumai 3 and a number of genotypes, including China 2, China 1, China 4 and China 7. China 6 and China 8 again displayed moderate values, and China 3 and China 5 were grouped with the susceptible controls Rialto and Bobwhite. In 2006/2007, similar patterns were seen (Tables 4.19 and 4.20). Sumai 3 remained one of the most resistant genotypes, with the addition of USA1 and CIMMYT 2. For the percentage ears, China 1, China 8 and China 2 all grouped with Sumai 3. China 6, China 4, China 3, and China 5 all group together in the middle of the Table, suggesting moderate levels of resistance. For the percentage spikelets, no significant difference was seen between Sumai 3 and any of the Chinese genotypes. In summary, good levels of type I resistance not significantly different from Sumai 3 were seen in Ning 7840, USA1, CIMMYT 2, China 8, and China 1 for one year and Wuhan and China 2 for two years. Levels of type II resistance were significantly similar to Sumai 3 in Ning 7840, USA1, CIMMYT 2, China 8, and China 1 for one year and Wuhan and China 2 for two years. The AUDPC of percentage ears was low in Ning 7840, Wuhan, Gottingen 2, China 1, and China 8 for one year and China 2 for two years. The AUDPC of percentage spikelets was low in China 1, China 2, China 4, and China 7 for one year only.

4.3.5. Measurement of DON mycotoxin levels in harvested grain

According to the theory presented in section 4.1, genotypes showing low AUDPC values should have low DON accumulation. The DON content was measured in harvested grain using GC-MS. Wide ranges of DON contents for the various genotypes were seen in both years, with 1749 - 11751 ppb in 2005/2006 and 576 - 11417 ppb in 2006/2007 (Tables 4.21 and 4.22). In both years NIV and the acetylated derivatives of DON, 3-ADON and 15-ADON, were

Table 4.19 AUDPC Ears (2006/2007)

Code	Genotype	AUDPC (Ears)	Rank AUDPC (Ears)
14	USA1	1436	1
19	CIMMYT 2	1544	2
11	Sumai 3	1757	3
13	Wuhan	3014	4
30	**China 2**	3117	5
29	Alsen	3139	6
32	**China 8**	3512	7
28	**China 1**	3646	8
17	Gottingen 3	6014	9
26	China 7	6086	10
12	Ning 7840	6725	11
27	**China 6**	7343	12
25	**China 4**	8481	13
24	**China 3**	9701	14
31	**China 5**	11123	15
10	Frelon	11147	16
18	CIMMYT 1	11396	17
23	Bobwhite	11780	18
15	Bobwhite	14153	19
16	Gottingen 2	15325	20
2	Petrus	19820	21
22	Paragon	23788	22
6	Consort	23965	23
7	Rialto	25803	24
	SED	2147.7	

Table 4.20 AUDPC Spikelets (2006/2007)

Code	Genotype	AUDPC (Spikelet)	Rank AUDPC (Spikelet)
11	Sumai 3	98	1
14	USA1	98	2
19	CIMMYT 2	101	3
13	Wuhan	178	4
30	**China 2**	196	5
32	**China 8**	238	6
29	Alsen	251	7
28	China 1	303	8
17	Gottingen 3	466	9
26	China 7	469	10
12	Ning 7840	515	11
27	**China 6**	615	12
25	**China 4**	736	13
31	**China 5**	793	14
24	**China 3**	920	15
18	CIMMYT 1	1012	16
10	Frelon	2522	17
23	Bobwhite	2975	18
16	Gottingen 2	3077	19
15	Bobwhite	3608	20
2	Petrus	5977	21
7	Rialto	7117	22
22	Paragon	7735	23
6	Consort	8262	24
	SED	862.6	

Table 4.21 DON content of harvested grain (2005/2006)

Code	Genotype	DON (ppb)	Ln (DON)	Rank Ln (DON)
12	Ning 7840	1995	6.982	1
13	Wuhan	1797	7.157	2
11	Sumai 3	1749	7.239	3
28	**China 1**	2451	7.465	4
16	Gottingen 2	3023	7.685	5
27	**China 6**	3014	7.790	6
14	USA1	4835	7.982	7
19	CIMMYT 2	3193	7.998	8
30	**China 2**	4875	8.230	9
17	Gottingen 3	4354	8.263	10
31	**China 5**	5110	8.284	11
10	Frelon	4563	8.300	12
29	Alsen	4575	8.332	13
18	CIMMYT 1	4904	8.370	14
24	**China 3**	5673	8.377	15
2	Petrus	5274	8.403	16
15	Bobwhite	5024	8.471	17
26	**China 7**	5826	8.531	18
23	Bobwhite[1]	8355	8.964	19
32	**China 8**	9031	8.968	20
7	Rialto	10170	9.001	21
25	**China 4**	9655	9.049	22
6	Consort	11337	9.309	23
22	Paragon	11751	9.355	24
	SED		0.6382	

Table 4.22 DON content of harvested grain (2006/2007)

Code	Genotype	DON (ppb)	Ln (DON)	Rank Ln (DON)
11	Sumai 3	578	5.727	1
26	China 7	576	5.775	2
13	Wuhan	719	6.340	3
19	CIMMYT 2	1168	6.389	4
14	USA1	1230	6.778	5
32	**China 8**	1195	6.845	6
29	Alsen	1918	6.910	7
30	**China 2**	1504	7.094	8
17	Gottingen 3	1725	7.107	9
28	**China 1**	2276	7.148	10
23	Bobwhite	2494	7.455	11
2	Petrus	2227	7.650	12
6	Consort	2964	7.740	13
12	Ning 7840	3245	7.828	14
10	Frelon	3123	7.830	15
15	Bobwhite	3735	7.957	16
16	Gottingen 2	3578	8.001	17
22	Paragon	3827	8.162	18
7	Rialto	4677	8.360	19
18	CIMMYT 1	4853	8.388	20
31	**China 5**	5524	8.458	21
27	**China 6**	9791	8.933	22
25	**China 4**	10683	8.947	23
24	**China 3**	11417	9.009	24
	SED		0.5546	

not detected in any samples. In 2005/2006, Sumai 3 had a mean DON level of 1749 ppb, and Ning7840, Wuhan, China 1, Gottingen 2, China 6, USA1, and CIMMYT 2 all had statistically similar DON levels. Surprisingly, DON levels in China 2 were significantly higher. China 2 is the Sumai 3 provided from the Chinese breeders, and so should behave in a similar manner to the Sumai 3 included in this trial as the resistant control. China 5 and China 3 had comparable levels of DON to China 2. China 7, China 8 and China 4 all had high DON levels. In 2006/2007, Sumai 3 had the lowest DON levels, and in this year, the levels were significantly lower than the other genotypes tested. China 1, China 2 and China 8 all had low DON levels, whereas the remaining Chinese genotypes all had high DON contents. The susceptible genotypes demonstrated lower levels of DON accumulation than expected based on the data from 2005/2006. Disease levels were high in the field and high levels of DON were expected. It may be possible that these highly infected grain were lost during the mechanical threshing due to their light, shrivelled nature.

4.3.6. Measurement of pink and shrivelled grain

Following discussion with Prof. Á. Mesterházy, whilst attending the 9th European Fusarium Seminar, Wageningen (September 2006), the percentage of pink and shrivelled grain was measured in grain before milling in the 2006/2007 field season. Previous data suggested that these disease measurements had the best relationship with the final mycotoxin content in harvested grain. Grain was scored by eye using the scoring system shown in Figure 4.6 using the entire grain sample from each plot. The results are given in Tables 4.23 and 4.24. The percentage of pink grain ranged from 0 - 31.67% and the percentage of shrivelled grain ranged from 34.2 - 96.7%. Low values in both cases were seen in USA1, CIMMYT 2 and Gottingen 3.

4.3.7. Comparison of disease parameters and regression analysis

The contamination of grain with the DON mycotoxin is expected to be in approximate proportion to the amount of disease symptoms seen unless other mechanisms are in operation which alter this general relationship. According to

Figure 4.6 Visual assessment of pink and shrivelled grain. A-C Percentage of shrivelled grain. A represents 5% shrivelled grain, B represents 50% shrivelled grain and C represents 100% shrivelled grain. D-F Percentage of pink grain. D represents 1% shrivelled grain, E represents 5% shrivelled grain and F represents 50% shrivelled grain.

Table 4.23 Percentage of pink grain in harvested grain (2006/2007)

Code	Genotype	% Pink Grain	Logit % Pink Grain	Rank Logit % Pink Grain
17	Gottingen 3	0.00	-2.654	1
12	Ning 7840	0.17	-2.562	2
14	USA1	0.17	-2.562	3
19	CIMMYT 2	0.17	-2.562	4
26	China 7	-0.11	-2.557	5
30	**China 2**	1.00	-2.358	6
16	Gottingen 2	3.33	-2.326	7
11	Sumai 3	1.83	-2.299	8
2	Petrus	0.67	-2.285	9
6	Consort	1.17	-2.265	10
13	Wuhan	2.00	-2.207	11
28	**China 1**	2.00	-2.207	12
23	Bobwhite	2.17	-2.115	13
29	Alsen	7.00	-2.061	14
32	**China 8**	3.33	-1.984	15
10	Frelon	4.67	-1.648	16
15	Bobwhite	5.50	-1.590	17
7	Rialto	9.33	-1.541	18
25	**China 4**	15.17	-1.465	19
24	**China 3**	17.50	-1.309	20
22	Paragon	13.50	-1.301	21
27	**China 6**	20.00	-0.759	22
31	**China 5**	20.83	-0.730	23
18	CIMMYT 1	31.67	-0.663	24
	SED		0.3852	

Table 4.24 Percentage of shrivelled grain in harvested grain (2006/2007)

Code	Genotype	% Shrivelled Grain	Logit % Shrivelled Grain	Rank Logit % Shrivelled Grain
14	USA1	34.2	-0.837	1
29	Alsen	42.5	-0.273	2
19	CIMMYT 2	49.3	-0.093	3
2	Petrus	64.2	0.245	4
28	**China 1**	53.3	0.261	5
11	Sumai 3	63.3	0.345	6
17	Gottingen 3	60.0	0.425	7
32	**China 8**	71.7	0.598	8
25	**China 4**	75.0	0.637	9
16	Gottingen 2	71.7	0.732	10
30	**China 2**	66.7	0.762	11
27	**China 6**	75.8	0.834	12
15	Bobwhite	78.3	0.899	13
31	**China 5**	71.7	0.906	14
24	**China 3**	73.3	0.941	15
12	Ning 7840	89.2	1.045	16
10	Frelon	75.8	1.059	17
13	Wuhan	75.8	1.202	18
6	Consort	91.7	1.262	19
23	Bobwhite	86.7	1.294	20
18	CIMMYT 1	92.5	1.317	21
26	China 7	93.6	1.400	22
7	Rialto	88.3	1.473	23
22	Paragon	96.7	2.033	24
	SED		0.6442	

the theory presented in section 4.1, the AUDPC values should have a good relationship with final content. Regression analyses were carried out using simple linear regression on the data to investigate the relationship between the accumulation of DON mycotoxin in the grain and the visible disease symptoms. Adjusted R^2 values are given in Table 4.25 with the standard errors for these values. In 2005/2006, the strongest associations were between DON and the percentage of spikelets infected. The percentage of ears infected was also related to the DON content, but not strongly. The relationship between AUDPC and DON was not significant for both percentage ears and percentage spikelets, suggesting that the DON accumulation in the grain cannot be predicted from the AUDPC levels. In 2005/2006, weak, but significant relationships were seen between DON and percentage ears and percentage spikelets. In 2006/2007, the strongest association was observed between DON and the percentage of pink grain. Moderate, but significant, relationships were seen for the percentage ears, percentage spikelets and AUDPC ears and spikelets. Over the two years, the best relationship to DON was with the percentage of pink grain. To investigate these relationships further the DON data was plotted against both the percentage spikelets and percentage ears for 2005/2006 (Figures 4.7 and 4.8). This was not repeated for the 2006/2007 data because the DON data displayed some anomalies in the known susceptible cultivars and these were suspected to have occurred post harvest. If the DON content in the grain was related solely to the percentage spikelets infected (Figure 4.7), there should be a parallel relationship between the two parameters. However, China 4, China 8 and Paragon all display higher levels of DON than predicted by the visible symptom scores, whilst China 6, China 5 and Bobwhite all generated lower DON levels than expected. These same trends are not as obvious for the percentage ears infected (Figure 4.8), and this is supported by the lower adjusted R^2 value in the regression analysis (Table 4.25).

Table 4.25 Summary of regression analyses between DON content and measured disease parameters

	Percentage variance accounted for	Standard Error
2005/2006		
Percentage Ears	31.6	0.721
Percentage Spikelets	34.0	0.714
AUDPC Ears	n/s	
AUDPC Spikelets	n/s	
2006/2007		
Percentage Ears	49.4	0.890
Percentage Spikelets	48.2	0.896
AUDPC Ears	53.4	0.867
AUDPC Spikelets	51.9	0.880
Percentage Pink Grain	53.8	0.865
Percentage Shrivelled Grain	n/s	

n/s = not significant at p = 0.05

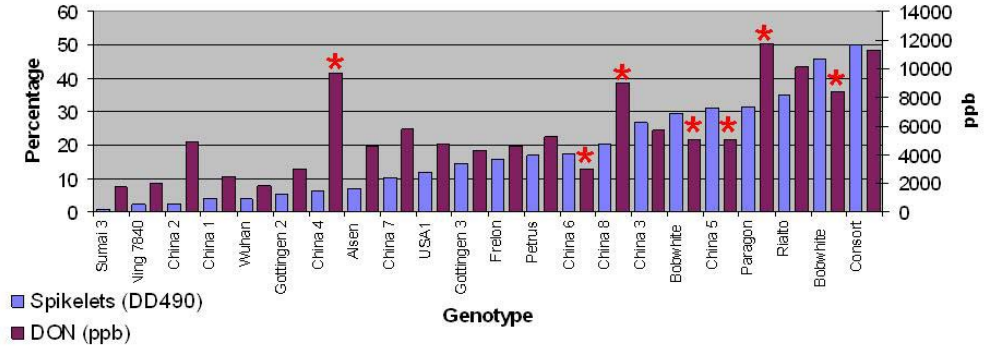


Figure 4.7 Percentage of spikelets infected and final DON content of harvested grain for the 24 tested genotypes (2005/2006). Asterisks indicate potentially anomalous DON results where DON differs to that expected for visible disease scores.

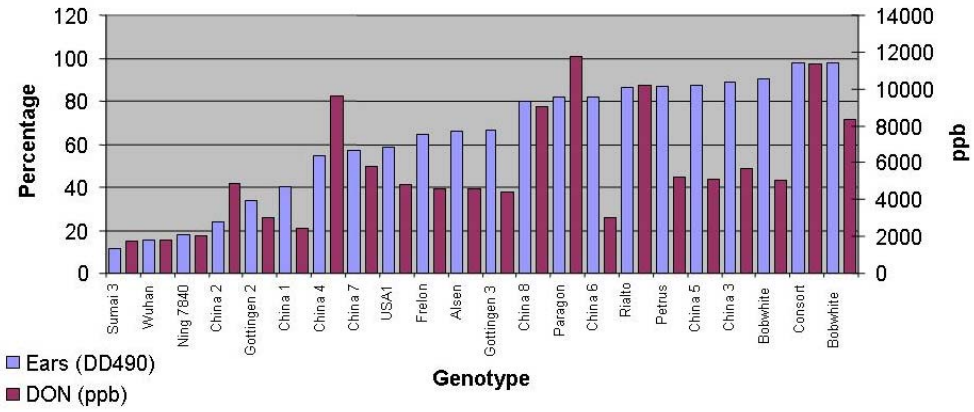


Figure 4.8 Percentage of ears infected and final DON content of harvested grain for the 24 tested genotypes (2005/2006).

4.3.8. Cross-year comparison

Genotype ranking of the different disease measurements in the two years shows good general agreement (Table 4.26). Deviations include Gottingen 2, Ning 7840, and China 8. Gottingen 2 is a tall genotype, and as such could have escaped proper inoculation. Ning 7840 appeared more susceptible in 2006/2007, which is likely due to seed mix-up at the seed bulking stage in the glasshouse. China 8 appears to perform better in 2006/2007.

4.3.9. Quantification of DON-3-*O*-glucoside

During the field season 2005/2006, Lemmens *et al*. (2005) reported that the 3BS QTL (now designated *Fhb1*) may function in the detoxification of DON to DON-3-*O*-glucoside. A few of the genotypes tested in the first year of the full field trial showed potential signs of abnormal DON accumulation. To determine whether a similar detoxification reaction was taking place in these genotypes, eight genotypes were sent to Prof. M. Lemmens for further analysis. The genotypes chosen are given below in Table 4.27. Sumai-3 was included as the positive control, and Paragon as the negative control. Although CIMMYT 1 and CIMMYT 2 were not included in the 2005/2006 trials, previous data has suggested these two genotypes had a high level of resistance (Daudi, 2007). China 5 displayed lower levels of DON than expected, and China 8 displayed more DON than expected, and therefore provided an interesting comparison. Alsen showed moderate levels of resistance and contains Sumai 3 in the pedigree. Two controls were included by Prof. Lemmens, the isogenic lines E1_86U which lacks *Fhb1* and E2_1T which contains *Fhb1*. The method used follows that detailed previously (Lemmens et al., 2005). Briefly, four florets were selected from the outer segments of two spikelets in the centre of a single ear. These florets were each injected with 20 µl of DON at 10g/l between the palea and lemma. This was followed by a repeat dose 24h later of 20 µl of DON at 2 g/l in each spikelet. Ears were allowed to develop to ripening before harvesting. DON and DON-3-*O*-glucoside concentrations were calculated using this material and are given in Table 4.27. The amount of DON and DON-3-*O*-glucoside recovered at the end of the experiment (five weeks post inoculation)

Table 4.26 A cross-year comparison of ranks of the disease parameters and plant measurements

Code	Genotype	Logit (Spikelet DD310) 2005/2006	Logit (Spikelet DD280) 2006/2007	Logit (Spikelet DD490) 2005/2006	Logit (Spikelet DD520) 2006/2007	AUDPC (Spikelet) 2005/2006	AUDPC (Spikelet) 2006/2007	Logit (Ears DD310) 2005/2006	Logit (Ears DD280) 2006/2007	Logit (Ears DD490) 2005/2006	Logit (Ears DD520) 2006/2007
2	Petrus	14	16	13	21	14	21	15	21	21	21
6	Consort	24	24	24	22	24	24	24	23	24	22
7	Rialto	21	23	22	23	20	22	23	24	19	24
10	Frelon	8	6	12	20	12	17	8	3	12	20
11	Sumai 3	1	4	1	5	1	1	1	1	1	4
12	Ning 7840	2	1	3	12	5	11	2	6	3	16
13	Wuhan	4	5	2	3	3	4	3	9	2	7
14	USA1	7	8	8	1	11	2	6	2	9	1
15	Bobwhite	18	19	20	18	19	20	18	16	20	18
16	Gottingen 2	3	15	5	17	4	19	5	15	5	19
17	Gottingen 3	11	2	11	14	8	9	13	7	11	15
18	CIMMYT 1	22	20	17	13	17	16	22	20	22	14
19	CIMMYT 2	16	3	15	2	15	3	14	4	15	2
22	Paragon	15	22	19	24	18	23	19	22	16	23
23	Bobwhite[1]	19	12	23	19	23	18	20	12	23	17
24	**China 3**	23	21	18	15	22	15	21	18	18	12
25	**China 4**	10	13	7	11	7	13	10	14	7	10
26	China 7	13	17	10	8	9	10	9	17	8	8
27	**China 6**	17	14	14	10	13	12	16	13	14	11
28	**China 1**	12	11	6	7	6	8	12	11	6	6
29	Alsen	6	9	9	9	10	7	7	5	10	9
30	**China 2**	5	7	4	4	2	5	4	8	4	5
31	**China 5**	20	18	21	16	21	14	17	19	17	13
32	**China 8**	9	10	16	6	16	6	11	10	13	3

Table 4.26 continued

Code	Genotype	AUDPC (Ears) 2005/2006	AUDPC (Ears) 2006/2007	Ln (DON) 2005/2006	Ln (DON) 2006/2007	Plant Height (cm) 2005/2006	Plant Height (cm) 2006/2007	Pedun. Length (cm) 2005/2006	Pedun. Length (cm) 2006/2007	Logit % Pink Grain	Logit % Shrivelled Grain
2	Petrus	14	21	16	12	22	21	15	2	9	4
6	Consort	24	23	23	13	6	13	4	4	10	19
7	Rialto	22	24	21	19	19	17	3	1	18	23
10	Frelon	11	16	12	15	16	16	8	7	16	17
11	Sumai 3	1	3	3	1	20	19	24	24	8	6
12	Ning 7840	2	11	1	14	17	23	21	18	2	16
13	Wuhan	3	4	2	3	14	12	20	20	11	18
14	USA1	9	1	7	5	18	18	17	16	3	1
15	Bobwhite	18	19	17	16	8	9	14	14	17	13
16	Gottingen 2	5	20	5	17	24	24	23	13	7	10
17	Gottingen 3	8	9	10	9	23	22	16	5	1	7
18	CIMMYT 1	19	17	14	20	13	15	2	23	24	21
19	CIMMYT 2	15	2	8	4	5	7	1	10	4	3
22	Paragon	20	22	24	18	21	20	13	3	21	24
23	Bobwhite[1]	21	18	19	11	2	11	5	15	13	20
24	**China 3**	23	14	15	24	4	1	12	11	20	15
25	**China 4**	7	13	22	23	10	4	9	8	19	9
26	China 7	10	10	18	2	11	6	19	19	5	22
27	**China 6**	16	12	6	22	7	5	10	9	22	12
28	**China 1**	6	8	4	10	12	8	11	12	12	5
29	Alsen	12	6	13	7	15	10	22	21	14	2
30	**China 2**	4	5	9	8	9	14	18	22	6	11
31	**China 5**	17	15	11	21	1	3	6	6	23	14
32	**China 8**	13	7	20	6	3	2	7	17	15	8

Table 4.27 Quantification of DON and DON-3-*O*-glucoside

Genotype	Known / Predicted Resistance	Final Conc. DON (mg/kg)[a]	Final Conc. DON-3-O-glucoside (mg/kg)[a]	Ratio DON-3-O-glucoside/ DON	DON + DON-3-*O*-glucoside (mg/kg)
Sumai 3	Resistant	104	840	8.09	944
CIMMYT 1	Resistant	461	397	0.86	857
CIMMYT 2	Resistant	118	803	6.78	922
Alsen	Moderately resistant	470	837	1.78	1306
Bobwhite	Susceptible, lower DON than expected	382	306	0.80	688
China 8	Higher DON than expected	1445	710	0.49	2155
China 5	Lower DON than expected	807	410	0.51	1217
Paragon	Susceptible	231	380	1.64	611
E1_86U	-*Fhb1*	890	963	1.08	1853
E2_1T	+*Fhb1*	66	2250	34.2	2316

[a] The values presented are based on the mean of 10 ears per genotype

varied amongst the genotypes. This was not always related to the known and predicted resistance. Low levels of DON were seen for resistant genotypes Sumai 3, CIMMYT 2, E2_1T, and in the susceptible controls Paragon and Bobwhite. Moderate to high levels were recovered from China 8, China 5, and E1_86U, suggesting that the DON has not been metabolised in these genotypes. China 5 and China 8 produce lower or higher amounts of DON than expected from visible disease symptoms, as evidenced in the field trials. In this experiment, the amount of DON recovered was lower in China 5 than China 8, confirming this result. However, the amount of DON recovered from China 5 was only slightly lower than the susceptible experimental control E1_86U, suggesting that this resistance mechanism is not that strong. Levels of DON-3-*O*-glucoside again differed between the genotypes, and the highest levels were seen in the *Fhb1* containing E2_1T, moderate levels were seen in the *Fhb1* lacking E1_86U and Alsen, and the *Fhb1* containing Sumai 3 and CIMMYT 2. The lowest levels were seen in the susceptible controls Bobwhite and Paragon. DON detoxification is characterised by a high ratio of DON-3-*O*-glucoside to DON, as seen in the control E2_1T. High ratios were seen in Sumai 3 and CIMMYT 2, higher than that of the E186U negative control; however these values were not as high as the E2_1T positive control. Low ratios were seen for the other genotypes, suggesting that there was no detoxification by this mechanism in genotypes which did not contain *Fhb1*. In the control genotypes E2_1T and E1_86U, the sum of DON and DON-3-*O*-glucoside was approximately 2000 mg/kg. Interestingly, only China 8 has a total quantity similar to this, the other genotypes from this field trial all have lower numbers, suggesting that DON has been metabolised using an alternative method.

4.4 Discussion

Two years of field trials were conducted to evaluate the resistance of eight Chinese genotypes under UK conditions. The weather conditions in the two seasons were very different, but overall, the data was reasonably consistent. Some variation in the data is to be expected, as environmental factors play a part in the development of this disease (Ruckenbauer et al., 2001). Minor modifications were made to the protocol to adapt to the different

conditions in 2006/2007, such as the use of plastic covers to protect the plots from rain. These allowed the successful inoculation of plots in what were difficult conditions. New GC/MS machinery was introduced also for 2006/2007, which allowed enhanced specificity for mycotoxin detection. Mist irrigation ensured the maintenance of humid conditions, essential for maximal disease development (Lacey et al., 1999). Interestingly, Lemmens et al. (2004) found that continued mist irrigation actually caused a reduction in DON content in comparison to non-irrigated plots, even though disease levels were high.

Both years were highly conducive to disease development. Disease scores across the genotypes varied widely from the susceptible to the highly resistant. In this experiment, resistance was measured by comparison with the resistant genotype Sumai 3. Other groups have suggested that resistant and moderately resistant genotypes show percentage of spikelets infected at below 10% and 20% respectively (Bai and Shaner, 1996; Bai et al., 2001b; Bai et al., 2003). By these criteria, in this two year trial the genotypes Sumai 3, Wuhan, Ning 7840, China 2, Gottingen 2, China 1, China 4, Alsen, USA1, CIMMYT 2, China 8, China 7, and China 6 were all deigned resistant. The genotypes Gottingen 3, Frelon and Petrus were all classed as moderately resistant.

Across both years, Sumai 3 was consistently one of the more resistant genotypes, along with Wuhan, Ning 7840, and China 2. Other genotypes, such as USA1, Gottingen 2, and China 8, were identified as resistant as Sumai 3 in only one year. High levels of resistance were consistently seen in China 1, although significantly lower than that of Sumai 3. China 1 does not contain any of the resistance QTL detailed in chapter 3, therefore this genotype could still provide an alternative source of resistance and could be used in pyramiding of resistance. Other potential sources of resistance for this purpose include China 4, China 6, China 7, China 8, and USA1, which all displayed good levels of resistance and did not contain any of the QTL. One of the best sources of resistance, both in the field and in the DON-3-*O*-glucoside assay is CIMMYT 2. It contains *Fhb1*, but it would be useful to examine the basis for this resistance further by mapping the QTL involved to confirm whether it is solely due to the *Fhb1* QTL. Interestingly, China 2 contained all 3 QTL, but displayed levels of

DON contamination significantly higher than Sumai 3. This suggests that other mechanisms involved in the reduction of DON contamination are present in Sumai 3 other than those conferred by the QTL on chromosomes 3BS, 5A, and 6BS. The selected susceptible control genotypes were consistently susceptible over both years. Bai and Shaner (1996), also found that rankings of genotypes over years was consistent for resistant and susceptible genotypes, but more varied for moderately resistant or moderately susceptible genotypes.

The relationship between disease levels and the *Rht* alleles was also investigated using these genotypes. A general trend was seen between the *Rht* allele combinations and actual plant height. The presence of specific *Rht* alleles is known to be associated with enhanced susceptibility. Therefore in the present study their presence was likely to influence the levels of resistance observed. Sumai 3 has the wild type, tall allele at *Rht-D1a*, which is associated with enhanced resistance, but at the *Rht-B1* locus, the situation is more complicated. The wild type allele is associated with enhanced type I resistance, but reduced type II resistance. High levels of type II resistance are seen in Sumai 3, so if the *Rht-B1b* allele was present instead of the wild type *Rht-B1a* allele, type II resistance levels could be even higher. Similarly, the high level of resistance seen in CIMMYT 2 is in addition to compensating for the susceptibility to initial infection associated with the chromosomal region harbouring *Rht-D1b*. For the susceptible control Bobwhite, the presence of *Rht-D1a* suggests that without this allele, levels of resistance could be even lower than those seen in the field trials.

Mycotoxin contamination reached similar levels in both years. The DON contents in the grain retrieved by mechanical threshing were, in general, higher than the EU permitted level for flour of 750 ppb. The 2005/2006 field season produced the higher DON contents, with no genotype containing DON levels lower than 750 ppb. In 2006/2007, Sumai 3 had DON levels significantly lower than 750 ppb. These high levels are to be expected under the artificial inoculation and continuous high humidity conditions. Although the inoculum concentration of 1.3×10^4 is lower than that applied by other groups

(Buerstmayr et al., 2003; Somers et al., 2003), it is still likely to be much higher than natural inoculum levels.

The percentage of variance accounted for by the different visible disease parameters and the DON content of harvested grain was low to moderate. According to the model in Figure 4.1, DON contamination could be related to the AUDPC values. This proved not to be the case, with no significant relationship detected in 2005/2006 and only a moderate relationship in 2006/2007. Other groups have noted that AUDPC is strongly related to DON content (Lemmens et al., 1997; Bai et al., 2001b; Brennan et al., 2007). Previous studies have demonstrated that the relationship between DON and FEB rating, either percentage ears, percentage spikelets, or more commonly a combination of the two, ranges from not significant through to strongly related. For example, DON content was strongly related to the FEB score in some experiments (Teich and Hamilton, 1985; Teich et al., 1987; Wang and Miller, 1988; Lemmens et al., 1997; Mesterhazy, 2002; Lemmens et al., 2004; Miedaner et al., 2004), moderate in others (Bai et al., 2001b; Miedaner et al., 2003b; Somers et al., 2003; Jiang et al., 2006a; Gosman et al., 2007), and in some cases poor or not significant (Martin and Johnston, 1982; Liu et al., 1997; Edwards et al., 2001; Ma et al., 2006b; Yu et al., 2008c). Fusarium damaged kernels, which can include the pink and shrivelled grain, were measured in the 2006/2007 field season. These are frequently referred to in the literature as a good prediction of the amount of DON in a sample (Martin and Johnston, 1982; Teich et al., 1987; Lemmens et al., 1997; Bai et al., 2001b; Mesterhazy, 2002; Abate et al., 2008). In this study, the relationship was significant, but only moderate. This is likely to be due to a number of factors. Firstly, DON levels for the susceptible controls Consort, Rialto and Paragon were much lower than expected based on the previous year's data, and considering the extent of the visible disease symptoms. This may have been due to the loss of highly infected, shrivelled grain during the mechanical threshing process. This grain would have contained the highest concentrations of DON and loss of this grain by this method has been well documented, therefore skewing the data (Schaafsma et al., 2001; Bai and Shaner, 2004; Yu et al., 2008c). This loss

may not have been seen if the samples were hand threshed. This would have been a more preferable method, however the large number of samples which were generated in this trial did not allow this approach. In order to hand thresh this number of samples would require more time and labour to complete.

The low to moderate regression values suggest that the amount of DON mycotoxin present is more than a function of visible disease in this group of genotypes. Therefore predictions of DON content based solely on visible symptoms are likely to be inaccurate. Other variables may alter the amount of DON mycotoxin produced such as moisture and other environmental factors (Miedaner and Perkowski, 1996), different resistance processes, such as resistance to the accumulation of DON, or the degradation of DON produced (Ma et al., 2006b). Indeed, some studies have revealed that the amount of fungus present in grain does not necessarily relate strongly to the amount of DON produced (Brennan et al., 2007), suggesting that other factors influence the accumulation of DON mycotoxin.

Previous studies have also found genotypes which contain DON levels different from that expected (Mesterhazy et al., 1999; Bai et al., 2001b; Miedaner et al., 2001; Bottalico and Perrone, 2002; Miedaner et al., 2003b; Miedaner et al., 2004; Gosman et al., 2007; Yu et al., 2008c). Explanations for the lower DON content include detoxification or decomposition (Miller et al., 1985; Mesterhazy et al., 1999; Lemmens et al., 2005). Alternatively, lower DON content may be found when grain are bleached not through fungal infection but through desiccation (Lemmens et al., 1997). Obstruction of the rachis prevents water and nutrients reaching spikelets above those diseased, and these spikelets bleach prematurely. Under field conditions, these bleached spikelets are hard to distinguish by eye from spikelets bleached due to fungal infection, and so the level of disease might be overestimated (Bai and Shaner, 1996; Lemmens et al., 2004). This is reflected by the mycotoxin contents in the grain. Grain within the drought-induced bleached spikelets contain <5 mg kg^{-1} DON, where as the fungus-contaminated grain contains between 1 and 600 mg kg^{-1} (Sinha and Savard, 1997).

During this analysis of field resistance, there are many sources of variability. Biological variability results from the differences between the genotypes in the accumulation of DON, and this is the variability of interest. Variability also arises throughout the DON measurement process, with potential error introduced at the milling, weighing, extraction, and measurement stages. This variability can be minimised as much as possible, however, there have to be some compromises. Detailed measurements using GC-MS frequently necessitate the use of technical replicates to limit the variability from the stages mentioned above, thereby giving more confidence in the biological variability. Usually, three replicates of each sample, in this case each plot, are analysed. In theory, all three measurements should be the same or similar, and any deviations from this indicate errors in the sample preparation. This can prove very costly however, and in this project would have increased the sample number from 144 to 432, plus the standards, which would have been too expensive. As a compromise, a bulked analysis was carried out in 2007 in addition to the standard individual plot method carried out in 2006. Samples were pooled from the plots to give measurements for each genotype. Following on from this, statistical analysis was completed, and on advice from the project statistician, this data set was not as useful as the individual plots. Therefore, a balance needs to be achieved between the technical power of the method and the statistical analyses that could be carried out.

To investigate the possibility of some genotypes detoxifying the DON mycotoxin, eight wheat genotypes were sent to Austria for analysis of DON-3-*O*-glucoside levels by Prof. M. Lemmens. These genotypes were selected on the basis of the 2005/2006 field trial results. Lemmens *et al*. (2005) showed that the 3BS QTL (now *Fhb1* - see Chapter 1) from Sumai 3 was involved in the detoxification of the DON mycoxotin to DON-3-*O*-glucoside. Most of the genotypes sent for analysis do not contain the 3BS QTL, as determined by the molecular marker analysis described in chapter 3. Several interesting results emerged from this analysis. Firstly, a total of approximately 2000 mg/kg of DON + DON-3-*O*-glucoside was expected from the genotypes. Only China 8 contained this amount. Absence of DON and DON-3-*O*-glucoside could be due

to irreversible conjugation to cell wall components or catabolisation (M. Lemmens, pers. comm.), further supported by the fact that in the plant cell, DON has been localised to the cell wall (Kang and Buchenauer, 2000a; Kang et al., 2008). Sumai 3 and CIMMYT 2 both contain *Fhb1* and have moderate ratios between DON and DON-3-*O*-glucoside, suggesting detoxification by the previously described mechanisms (Lemmens et al., 2005). The other genotypes lack the *Fhb1* QTL, and conversely, the detoxification ratios are low. However, the low levels of recovered DON at the end of the experiment suggest that there may be other detoxification mechanisms at work, or that the DON is catabolised to form other products which are not detected using the current methods. Alternatively, it may be possible that only some of the DON was taken up by the plant during the experiment. For this experimental set-up, there was previous evidence that DON was taken up by the plant due to the bleaching of both DON-inoculated spikelets and further non-inoculated spikelets (Lemmens et al., 2005). The potentially differing amounts of DON uptake could further complicate the final amount of DON-3-*O*-glucoside recorded because if only some of the DON was taken up, then there was only a limited amount to be converted to DON-3-*O*-glucoside. A further possibility exists where this method of detoxification can take place in genotypes without the *Fhb1* locus. The *Fhb1* locus has been identified in different mapping populations (summarised in Chapter 1), however the resistance to DON by conversion to DON-3-*O*-glucoside has only been demonstrated in one (Lemmens et al. 2005). It is possible that if the experiments of Lemmens et al. were repeated using different mapping populations, further loci involved in this conjugation could be identified, which could explain the presence of DON-3-*O*-glucoside in some of the genotypes in this study which do not contain *Fhb1*.

These field trials demonstrate a good method for identifying resistance to FEB in hexaploid wheat, genotypes with exceptional levels of resistance such as Sumai 3 are clearly separated from other resistant materials. On a practical scale, the field trial is very time-consuming, demanding individual plot inoculations, followed by specific assessment dates on a plot-by-plot basis. This method is adequate for a study of this size and requires only one person to

conduct the trials, but on a larger scale, such detailed analysis may not be suitable for screening many thousands of wheat genotypes, such as in a commercial breeding programme. The number of assessment time points could be scaled down to maybe four or five over the growing season, and individual plot spraying could be replaced by spraying of the entire trial if the genotypes under evaluation flowered at similar times. Evaluation of DON contamination is more difficult to predict, as seen by the moderate relationships seen between the visible disease scores and final DON content. The method of DON evaluation used in these trials, whilst more precise, can be prohibitively expensive for a smaller breeding company. The method is also time-consuming and technically demanding. Alternative methods, such as the use of the Ridascreen® Fast DON ELISA kit (R-Biopharm AG, Darmstadt, Germany) may be more practical.

In summary, wheat genotypes have been identified with good levels of resistance which are not related to Sumai 3. To date, only the *Fhb1* QTL has been closely associated with reduction in DON contamination, but the genotypes tested here have demonstrated that other reduction mechanisms are present. In a practical breeding programme these genotypes could be used in addition to well known sources such as Sumai 3 to introduce multiple resistance mechanism into adapted cultivars.

CHAPTER 5: A QUALITATIVE AND QUANTITIVE EXPLORATION OF EAR INFECTION IN SELECTED GENOTYPES USING TWO REPORTER STRAINS

5.1 Introduction

Detection and quantification of fungal biomass is a key strategy in understanding a plant-pathogen interaction. Visualisation of the interaction provides an insight into the route of entry by the pathogen and the subsequent growth and development *in planta*. Similarly, the quantification of the fungus in different tissues and over time may give further insight of the interaction. Earlier methods of fungal biomass quantification included the measurement of ergosterol and chitin levels (Plassard et al., 1982; Miller et al., 1985; Newell et al., 1987; Wang and Miller, 1988). These were relatively slow. Later, faster PCR detection methods were developed to detect different species (Schilling et al., 1996; Niessen and Vogel, 1997; Nicholson et al., 1998; Demeke et al., 2005; Klemsdal and Elen, 2006). Quantification of fungal biomass was possible using competitive PCR (Nicholson et al., 1997; Nicholson et al., 1998; Glynn et al., 2007), and later by the use of real time PCR (Waalwijk et al., 2004; Dyer et al., 2006; Leisova et al., 2006; Fredlund et al., 2008).

An alternative way to monitor fungal development is the use of reporter strains of the fungi of interest. Previously used reporter constructs included the *Escherichia coli lacZ* gene which encodes *β*-galactosidase (Lis et al., 1983). However, due to high background levels in some host systems, this method was later replaced by the use of firefly (*Photinus pyralis*) luciferase (Ow et al., 1986), *E. coli* β-glucuronidase (GUS) (Jefferson et al., 1986), and jelly fish (*Aequorea victoria*) green fluorescent protein (GFP) (Chalfie et al., 1994). The GUS gene-fusion system was developed by Jefferson et al. (Jefferson et al., 1986) and the *E. coli uidA* gene codes for the enzyme β-glucuronidase (GUS). This enzyme catalyses the cleavage of *β*-glucuronides, which in the case of the substrate 4-methylumbelliferyl *β*-D-glucuronide (MUG), yields the product methylumbelliferyl, which is fluorescent (Jefferson et al., 1986). The GUS

system has the advantage of low background levels in different organisms and tissues, therefore increasing sensitivity. Enzyme activity can be quantified using fluorometry or spectrophotometry and is typically done in microtitreplates. In addition, either intact tissues or tissue sections can be histochemically stained to visualise fungal biomass *in situ* (Jefferson et al., 1987; Roberts et al., 1989; Pritsch et al., 2001). The disadvantages of this system however are the necessary generation and characterisation of transgenic strains which are then subject to strict licensing conditions in the UK by DEFRA and the HSE. Overall though, the GUS system for the measurement of fungal biomass is simpler and quicker than quantitative PCR analysis (Oliver et al., 1993; Doohan et al., 1998). The GUS system allows both qualitative and quantitative analysis. The fungus can be assayed qualitatively, by staining with 5-bromo-4-chloro-3-inodyl *β*-D-glucuronide (X-gluc), and quantitatively, by incubating with 4-methylumbelliferyl *β*-D-glucuronide (MUG) and measuring the fluorescence.

Since the development of the GUS system, many fungi have been transformed to express the GUS enzyme including *Aspergillus nidulans* (Roberts et al., 1989), *Aspergillus niger* (Roberts et al., 1989), *Cladosporium fulvum* (Roberts et al., 1989), *Aspergillus oryzae* (Tada et al., 1991), *Pseudocercosporella herpotrichoides* (teleomorph *Oculimacula yallundae*) (Bunkers, 1991), *Claviceps purpurea* (Smit and Tudzynski, 1992), *Ustilago maydis* (Richard et al., 1992), *Cochliobolus heterostrophus* (Monke and Schafer, 1993), *Fusarium oxysporum* (Couteaudier et al., 1993), *Leptosphaeria maculans* (Oliver et al., 1993), *Pyrenopeziza brassicae* (Ashby and Johnstone, 1993), *Fusarium moniliforme* (Yates et al., 1999), and *Fusarium verticillioides* (Yates and Sparks, 2008).

The FEB-causing fungi *F. culmorum* and *F. graminearum* have both been transformed by others using GUS or GFP as reporter proteins to locate and quantify fusarium biomass as well as to investigate the expression of the genes involved in trichothecene biosynthesis. For example, *F. graminearum* has been transformed with the *GFP* gene expressed from the *Aureobasidium pullulans* translation elongation factor promoter to provide constitutive expression (Skadsen and Hohn, 2004). A *GFP* expressing strain of *F.*

graminearum which was mutated at the *Tri5* locus has also been used for microscopic analyses (Jansen et al., 2005). A *F. graminearum* strain has been engineered for expression of enhanced green fluorescent protein (eGFP) under the control of the *Tri5* gene promoter (Ochiai et al., 2007). This strain would normally produce 4-ANIV and 4,15-diANIV as the principal trichothecenes, differing to the strains under investigation here which principally produce DON and its acetylated derivatives. Strains of *F. culmorum* and *F. graminearum* have both been transformed with the *GUS* gene under constitutive control for use in evaluating fungicides and measuring fungal biomass levels in relation to the expression of PR genes (Doohan et al., 1998; Pritsch et al., 2001) and the *F. graminearum* strain GZ3639 was transformed with the *GUS* gene under the control of the *Tri5* gene promoter (Chen et al., 2000).

Previous studies on other pathosystems have indicated that measurements of fungal biomass using the GUS system can be related to host resistance (Oliver et al., 1993; de la Pena and Murray, 1994). To establish whether the resistance of the wheat genotypes used in this project was reflected in the fungal biomass and / or induction of mycotoxin production, two transgenic reporter strains were used. Both strains were developed in our laboratory from the sequenced strain PH-1 of *F. graminearum.* The characterised reporter strain G3 expresses the *GUS* gene constitutively under the control of the *Aspergillus nidulans* promoter *gpdA* (Dr. Martin Urban and Dr. Chris Bass, unpublished), whereas in the reporter strain GP17 the *GUS* gene is under the control of the *Tri5* promoter and was shown to only be expressed when DON mycotoxin production was induced. In GP17 the *GUS* reporter gene is adjacent to an intact copy of the *Tri5* gene at the *Tri5* cluster in the genome and still produces wild type levels of DON and 15-ADON (Bass and Urban, unpublished). Previous studies monitoring the expression of the *Tri5* gene in wheat have been carried out (Doohan et al., 1999; Voigt et al., 2007), but have relied on the use of RT-PCR, which necessitates the time-consuming extraction of RNA.

Seven of the wheat genotypes used in the field trials (see chapter 4) were selected based on the results of the 2005/2006 field trial. Sumai 3 and

Bobwhite were selected as resistant and susceptible controls, respectively. China 1, China 5, China 8, Alsen and Gottingen 2 all displayed interesting levels of resistance. In addition, CIMMYT 1 and CIMMYT 2 were included because previous studies in our laboratory have demonstrated that these also provide high levels of resistance (Daudi, 2007).

5.2 Materials and Methods

The nine wheat genotypes were grown in the category 2 containment glasshouse according to the conditions in chapter 2, i.e. 20 ºC day, 18 ºC night with a 16 hour day. The two fungal isolates detailed above along with the wild type PH-1 isolate were cultured according to the conditions detailed in chapter 2. Isolates were grown on SNA plates for 7 days under near-UV light. Ears for inoculation were selected from either the 1st, 2nd, or 3rd tillers of the plant. Inoculations were carried out daily over a period of 8 weeks. Within each daily batch, at least one Bobwhite ear was inoculated whenever possible. Point inoculations were carried out on the first flowering spikelets of each ear using plugs of agar (3mm) taken from near the growing edge of the cultures. The end of a glass pipette was broken off to form a sheath over a disarmed syringe needle, which allowed the selection and transfer of an agar plug into the floret. Each ear was inoculated a total of 4 times, with 2 spikelets being inoculated in 2 florets. Each inoculated spikelet was marked on the glumes with a black filter pen. The inoculated ears were enclosed in plastic freezer bags (Sainsburys Supermarkets Ltd) which contained a water soaked cotton wool ball (Safety First Aid Co Ltd). A wooden stake was attached to each inoculated tiller on the plant and went inside the bag to provide support. By this approach high humidity was achieved specifically in the vicinity of each ear. For the first 24 hours, a small black double thickness bag was placed over the freezer bag to provide conditions of darkness required for uniform spore germination (Prof. Kim Hammond-Kosack, pers. comm.). Freezer bags were removed 5 dpi and whole ears were removed at 8dpi, before being wrapped in aluminium foil and stored at -80 ºC.

Quantitative MUG assays were carried out based on the method originally devised by Jefferson et al. (1987). The inoculated spikelets, any

further spikelets above and below the point of inoculation which showed bleaching symptoms, and a further asymptomatic spikelet outside of this region were separately analysed. Awns were trimmed from the spikelets as necessary and spikelets were placed into individual round-ended 2 ml tubes (Sarstedt). The spikelets were freeze dried overnight before being transferred to a fresh tube. Three x 3mm ball bearings were added to each tube, along with 500 µl of the GUS extraction buffer (50 mM $NaH_2PO_4.H_2O$ pH 7.0, 1M dithiothreitol (DTT), 0.5M Na_2EDTA pH 8.0, 10% Sodium Lauryl Sarcosine, 10% Triton X-100). Spikelets were ground for 2 mins at 30 bps, reversing the sample rack halfway. The extracts were spun down for 5 mins at 13, 200 rpm and 50 µl of the supernatant was removed for analysis. The remaining supernatant was stored at -20 ºC for future reference. Different assessment time points were established for each reporter strain, namely; 5, 10, 15, 20, 25 minutes for the consGUS, reporter strain G3, inoculated spikelets and the PH-1 control and 1, 2, 3, 4, 5 hours for *Tri5*GUS, reporter strain GP17, inoculated spikelets and the PH-1 controls. Water inoculated controls were evaluated at both time points. Fluoroplates (Thermo Scientific, Waltham, MA, USA) were set up for each time point and contained 180 µl of stop buffer (0.2M Na_2CO_3) in 94 of the 96 wells. In the remaining two wells were placed 200 µl of the 1 µM and 100nM methylumbelliferone (MU) standards. A reaction plate consisting of 94 wells containing 120 µl of the MUG assay buffer (1mM methylumbelliferyl glucuronide dissolved in the GUS extraction buffer) in the corresponding wells to the sample wells of the time point plates. The remaining two wells were blank. The reaction started when 12 µl of the extract was transferred into the plate containing the MUG assay buffer. At each time point, 20 µl of the reaction was transferred to a time point plate, where the reaction was quenched. Fluorescence was measured using an FL_X 800 Microplate Fluorescence Reader (Bio-Tek Instruments Inc, Vermont, USA) with excitation at 360 nm and emission at 460 nm.

MU quantities were calculated for each spikelet by the following formula:

$$MU = \frac{\text{fluorimeter reading of sample} \times 1000}{\text{fluorimeter reading of 1000 nM standard}}$$

Final quantities of MU were given as the rate of MU production over the time course by calculation of the slope of the plotted time point values and were expressed as units of nmol MU min^{-1} or nmol MU $hour^{-1}$.
Statistical analysis was carried out using GenStat v11 (Payne et al., 2005).

5.3 Results

A selection of wheat ears from 9 different wheat genotypes were inoculated with either one of the two transgenic fungal strains, the PH-1 wild type strain of *F. graminearum* or mock inoculated with water. GUS activity was measured by the rate of production of methylumbelliferone (MU). For the consGUS strain, MU was measured at 5 minute time points, due to the high levels of GUS activity. The units were therefore expressed as nmol MU min^{-1}. The expression of *Tri5*GUS is weaker than that of the constitutive GUS (Bass and Urban, pers. comm.), and therefore lower levels of GUS activity were anticipated. For this reason, MU levels were measured at hourly time points, with the unit nmol MU $hour^{-1}$. The full data set generated was very large, and is therefore deposited separately with Prof. Kim Hammond-Kosack, Rothamsted Research. The MU production rates from the two inoculated spikelets were pooled for each ear and the average across all the ears inoculated for each strain per genotype was calculated. These average values are given in table 5.1. Analysis of further spikelets from these ears would have been attempted if there had been more time available.

Background levels of MU were investigated with the use of water mock-inoculated and PH-1 wildtype-inoculated control ears. The values of MU in ears inoculated with PH-1 ranged from 13.0 - 29.4 nmol MU min^{-1} and 11.8 - 49.0 nmol MU $hour^{-1}$. The water controls gave values of 9.2 - 34.0 nmol MU min^{-1} and 15.1 - 48.9 nmol MU $hour^{-1}$. In both cases, the figures are low and approximately similar. This indicates that the presence of the untransformed *F. graminearum* did not lead to a significant increase of the methylumbelliferyl glucuronide product. Also within the water inoculated wheat ear the overall background was low.

During the inoculation phase of this experiment, the development of visual symptoms on all the Sumai 3 ears was far higher than anticipated from

Table 5.1 MU production in wheat spikelets inoculated with either reporter strain, wild type PH-1, or water

Genotype	consGUS[2]	PH-1 Control[2]	Water[2]	*Tri5* GUS[3]	PH-1 Control[3]	Water[3]
Alsen[1]	126.3	22.6	16.8	26.8	13.2	24.3
Bobwhite	168.7	29.4	15.3	103.2	49.0	28.3
China 1	225.3	27.8	*	121.8	22.2	*
China 5	144.6	21.5	34.0	34.7	18.4	48.9
China 8	170.1	20.3	17.1	34.9	17.9	32.5
CIMMYT 1	136.8	15.0	27.0	44.3	13.4	28.0
CIMMYT 2	198.7	23.2	20.9	54.5	11.8	46.6
Gottingen 2	142.7	13.0	16.2	109.4	16.6	33.1
Unknown[4]	209.0	22.7	9.2	141.2	25.3	15.1

[1] The number of ears inoculated per strain – genotype combination ranged from 12 to 55 (see Tables 5.2 and 5.3). All spikelets were harvested 8 days post inoculation.
[2] Units given as nmol MU produced per minute. PH-1 and water controls carried out using 5 minute time points.
[3] Units given as nmol MU produced per hour. PH-1 and water controls carried out using 1 hour time points.
[4] This genotype was initially thought to be Sumai 3 but has since had to be classified as an unknown susceptible genotype with awns.
* Data not available

the field results previously obtained. This suggested that this genotype was not Sumai 3 and has been labelled as unknown.

To compare the levels of MU production between genotypes, analysis of variance was performed on the consGUS and *Tri5*GUS datasets, after transformation of the data with natural logarithms to ensure uniform variance. The results from these analyses are given in tables 5.2 and 5.3. No significant difference was found between genotypes inoculated with the consGUS *F. graminearum* strain. This indicates that the fungal biomass accumulation was similar in each genotype. Significant differences were found however for the *Tri5*GUS data. The lowest levels of induction were seen in Alsen, which was included due to previously identified moderate to high levels of type I and type II resistance, and China 8, which accumulated higher amounts of DON than expected based on the visual symptoms observed in field trials. China 5, which accumulated lower amounts of DON than expected, had similar levels of *Tri5* induction to China 8 whilst CIMMYT 1 and CIMMYT 2, both of which demonstrated good levels of resistance to visible symptoms in previous field trials and year 2 field trials in this project, had higher levels of induction. The highest levels of mycotoxin induction were detected in the susceptible genotype Bobwhite, the two moderately resistant genotypes China 1 and Gottingen 2 and the unknown.

5.4 Discussion

Assays using reporter strains, such as the GUS system have been used previously to identify sources of resistance to fungal diseases in wheat, for example to the eyespot fungus *Pseudocercosporella herpotrichoides* (teleomorph *Oculimacula yallundae*) (de la Pena and Murray, 1994). In these wheat – fusarium experiments, a GUS reporter gene was used to investigate previously identified resistant wheat genotypes for possible differences in the fungal biomass and for the induction of trichothecene biosynthesis. The transgenic strains harbouring the constitutive GUS construct (consGUS) provided a method to quantify fusarium biomass in wheat ears. No significant difference was found between any of the resistant and susceptible genotypes. This demonstrates that the resistance evident in some genotypes is

Table 5.2 Transformed data for consGUS inoculated spikelets

Genotype	Number of ears	nmolMU hour^{-1}	ln(nmolMU hour^{-1})
Alsen	12	147.0	4.694
Gottingen 2	16	142.7	4.793
CIMMYT 1	22	148.9	4.907
Bobwhite	55	177.8	4.913
CIMMYT 2	21	207.9	4.955
China 5	20	172.9	5.000
China 8	25	176.8	5.032
China 1	30	240.2	5.160
Unknown	22	227.9	5.394

Note: for this data set, SED values are not presented as P > 0.05 in the ANOVA.

Table 5.3 Transformed data for *Tri5*GUS inoculated spikelets

Genotype	Number of ears	nmolMU hour^{-1}	ln(nmolMU hour^{-1})
Alsen	14	26.8	3.250
China 8	22	34.9	3.392
China 5	22	34.7	3.450
CIMMYT 1	24	44.3	3.712
CIMMYT 2	18	54.5	3.925
Gottingen 2	18	109.4	4.224
Bobwhite	44	103.2	4.341
China 1	26	121.8	4.531
Unknown	18	141.2	4.942
SED			0.2024

not due to a reduction in fusarium biomass during the first eight days following infection of the assayed spikelets. Previous studies on fungal biomass levels in resistant and susceptible wheat cultivars revealed that biomass levels were higher in Sumai 3 than the susceptible cultivar Wheaton (Pritsch et al., 2001). Taken together, these results suggest that the role of fungal biomass in relation to visible disease development is small following point inoculation, and that the resistance mechanisms of the host is more important in determining the final disease levels of the host.

Significant differences between wheat genotypes were evident when the strain harbouring the *GUS* gene under the control of the *Tri5* gene promoter was used. This strain still produces DON mycotoxin in the wheat ear. The quantity of methylumbelliferone produced per hour shown here represents the induction of the *Tri5* gene (Dr. Martin Urban, pers. comm.) and ultimately represents the induction of DON biosynthesis. As the current experiments were carried out under controlled environment conditions, the differences in mycotoxin biosynthesis induction detected here are likely to be due to the genotype, and not differences in humidity or environmental temperature. Unlike the field trials which determined the final amount of DON in the harvested grains (see chapter 4) in this experiment, the levels of biosynthesis induction were measured. This permitted a finer dissection of the possible mechanisms that resulted in specific genotypes accumulating lower DON levels. By focusing solely on *Tri5* gene induction, this has eliminated the complications arising from detoxification, or resistance to accumulation of the mycotoxin. Also the interpretation of the results obtained was simplified because all the genotypes showed a similar level of fusarium biomass accumulation. Differences between the genotypes in *Tri5* induction suggest that the host environment differs, and in some genotypes the tissue is more conducive to sustained gene expression whereas in others expression is limited. Previous work has demonstrated that DON biosynthesis may be influenced by carbon and nitrogen sources (Miller and Greenhalgh, 1985; Jiao et al., 2008), grain type (O'Neill et al., 1993), reactive oxygen species (Ponts et al., 2003; Ponts et al., 2006; Ochiai et al., 2007; Ponts et al., 2007), levels of sodium chloride (Ochiai et al., 2007) and the

presence of magnesium (Pinson-Gadais et al., 2008). Possibly either constitutive or interaction induced biochemical differences in the interior of the wheat floret between the genotypes may play an important role in the early induction and/or suppression of DON mycotoxin production.

Relating these data to the previous field trials and marker work, China 1 displayed high levels of field resistance, and did not contain any of the resistance QTL. A comparatively high level of mycotoxin induction seen in these experiments, coupled with low to moderate levels of mycotoxin accumulation in the field trials suggest that the resistance observed in this genotype may be based on the degradation of the mycotoxin in addition to a reduction in visible symptoms via a mechanism different to that associated with *Fhb1*. The lowest levels of mycotoxin biosynthesis induction were seen in Alsen, which is related to Sumai 3. Three of the four diagnostic marker alleles for *Fhb1* were identified in Alsen, suggesting the presence of *Fhb1*, the principal locus associated with degradation of DON. Together, these two mechanisms may be sufficient to lower DON levels in harvested grain. Bobwhite and China 5 were highlighted for a lower accumulation of DON than expected in harvested grain during year 1 of the field trials. No resistance QTL were present in these genotypes. In these experiments, China 5 demonstrated low levels of DON induction, which may explain the low DON contents seen after field experiments. Bobwhite in contrast displayed comparatively higher levels of DON induction, suggesting that the low DON content in the field was not due to a low level of induction of DON biosynthesis, and therefore the presence of a further resistance mechanism. China 8 was highlighted for an increased production of DON. In this assay, the values for DON induction were similar to those for China 5, which suggests that after an initial low level of induction of DON biosynthesis, DON content increases rapidly. In CIMMYT 2, low levels of DON are seen in harvested grain. *Tri5* expression levels are moderate in this genotype, which may not be enough to reduce DON levels to those seen in the field. In comparison to other genotypes in this experiment, lower levels of DON are seen in field trials, but with a higher level of induction, suggesting that the presence of *Fhb1* in this genotype may be more influential in reducing the final

DON content. CIMMYT 1 was included for similar reasons to CIMMYT 2, but does not contain *Fhb1*. Levels of *Tri5* expression are similar between the two genotypes, but the DON accumulation in grain from the field trials is higher in CIMMYT 1, suggesting that the absence of *Fhb1* is the main cause for the difference.

The positive control for this experiment was Sumai 3. However, phenotypic data from this experiment and others (data not shown) showed that this genotype was not Sumai 3, and was most likely due to a mix up in seed production in the glasshouse. As such this data set will not be discussed further. Molecular marker analysis of this genotype could have confirmed the suspected absence of the three Sumai 3 resistance QTL, as detailed in Chapter 3, had time allowed. A further experiment has since been done to compare just Bobwhite and a verified source of Sumai 3, and another member of the laboratory will complete the MUG assays to finalise the datasets.

CHAPTER 6: FURTHER EXPLORATION OF THE LEAF BIOASSAY

6.1 Introduction

Field trials to screen potential wheat lines for resistance to FEB are expensive and time consuming. One of the current priorities for breeders is to develop a screening assay which reduces the time and labour involved in conventional resistance screening. Previously identified assays have focused predominantly on seed germination assays. Various assay methods have been devised including the germination of wheat seeds on mycelial homogenate-covered PDA plates (Brennan et al., 2003; Brennan et al., 2007) or alternatively imbibing wheat seeds in a conidial suspension of a number of *Fusarium* and *Microdochium* spp. (Browne and Cooke, 2005), before measuring the subsequent coleoptile growth. Significant positive correlations between the coleoptile growth and FEB symptoms or disease ratings under controlled environment conditions have been obtained between the *in vitro* seedling assay and AUDPC (r = 0.584) and coleoptile retardation and DON content (r = 0.488) (Brennan et al., 2007). Browne (2007) also found that in seedling assays inoculated with *Microdochium majus* (a non-mycotoxin producer) there was a correlation with FEB disease incidence (r = -0.41), severity (r = -0.47), index (r = -0.46) and DON content (r = -0.40) caused by *F. graminearum*. Assays for seedling resistance have also been significantly correlated with visual FEB ratings from the UK recommended list (r = 0.45) (Browne and Cooke, 2005). However, although these correlations were all significant, the low r values suggest that the relationships may not be strong enough to use these assays as screening tools. Other coleoptile assays have involved the use of Fusarium metabolites including DON, though distinguishing between resistant and susceptible genotypes was not consistent (Wang and Miller, 1988; Bruins et al., 1993). These assays provide potential methods to screen for resistance in reduced time scales compared to field screening. The relationships were moderate, but significant and the assay with the most promise was the seedling assay developed by Brennan et al. (Brennan et al., 2007). However, the assay

was based on only a limited number of wheat genotypes and the authors expressed caution in interpreting the results with respect to devising a screening assay.

More recently, focus has turned to the use of wheat seedlings in leaf assays. A detached leaf assay was developed by Diamond and Cooke (1999), which used leaf segments placed on water agar and then inoculated with *M. nivale* or *M. majus* (formerly *M. nivale* var. *nivale* and var. *majus*). Incubation period (the time taken from inoculation to symptom development) and latent period (the time taken from inoculation to conidia production) and latent periods were measured as well as lesion size (Diamond and Cooke, 1999; Browne and Cooke, 2004). Initial experiments with this assay demonstrated a good correlation between ear incubation period (the time taken from inoculation to symptom development) and leaf assay incubation period (r = 0.820, p = 0.02) (Diamond and Cooke, 1999). Later experiments demonstrated a further good correlation between latent period and recommended list ratings in European cultivars (r = 0.70; P < 0.001) (Browne and Cooke, 2004). However, when exotic sources of resistance such as Sumai 3 were explored, correlations were reversed and the relationship between FEB susceptibility and latent period (r = 0.53; P < 0.01) showed that genotypes such as Sumai 3, which were resistant to ear infection, were highly susceptible in the leaf assay. The authors suggested that this discrepancy arose because Sumai 3 does not possess resistances detected by the leaf assay (Browne and Cooke, 2004). Further use of the *Microdochium*-based leaf assay on wheat material from the USA gave contrasting results, with longer incubation periods relating to higher disease levels (Browne et al., 2005). Brennan et al. (Brennan et al., 2005) found no significant correlation between leaf reaction and FEB symptoms under glasshouse conditions. Taken together, these results suggest that, whilst showing potential, this assay is not suited to discriminating between resistant genotypes from a wide range of geographical origins.

The Rothamsted Research group has previously developed an attached wheat leaf bioassay using the mycotoxin producing species *F. graminearum* and *F. culmorum* (Figure 6.1 and Figure 6.2). The susceptible wheat cultivar

Figure 6.1 Lesion on wheat cv. Bobwhite at 5 days post inoculation with *F. graminearum* PH-1

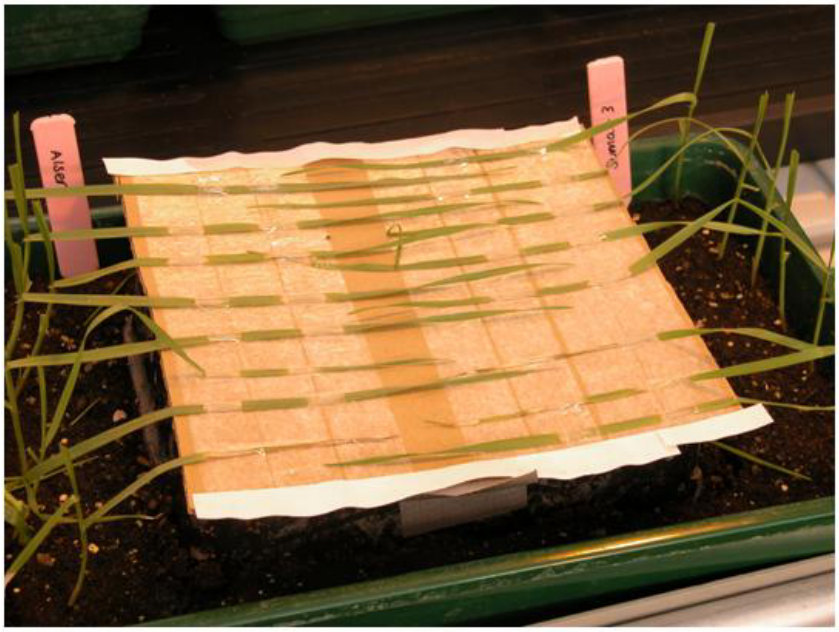


Figure 6.2 The attached leaf bioassay. Second leaves from two week old wheat seedlings are folded over an adhesive platform before being wounded with a syringe needle and inoculated with 2.5 x 10^5 conidia/ml *Fusarium graminearum* or *Fusarium culmorum* conidia suspension or sterile water.

Bobwhite, resistant breeding line Sumai 3 and six other resistant and moderately susceptible genotypes were explored. Significant difference in the rate of leaf lesion expansion and final lesion size were observed between Bobwhite and Sumai 3 and most of the other sources of resistance that were identified (Daudi, 2007). Initial experiments in this project aimed to confirm the ability of the leaf bioassay to predict resistance of wheat genotypes, which primarily focused on the screening of the Chinese genotypes and Sumai 3. Following on from this, the infection process was explored using electron microscopy. The final aim of this study was to investigate the role of DON in the development of lesions. This latter information was considered to be vital because in wheat ears mycotoxin production is required for full Fusarium virulence (Proctor et al., 1995a). Also, altered disease symptoms are evident when mycotoxin production is prevented through the targeted deletion of the *Tri5* gene which codes for trichodiene synthase, the first enzyme in the DON biosynthetic pathway (Bai et al., 2001a; Cuzick et al., 2008a). A mutant of the sequenced *F. graminearum* strain PH-1 at the *tri5* locus was used to investigate this (Cuzick et al., 2008a). Other strains used in these studies were the PH-1 wild-type strain, the UK *F. culmorum* isolate UK-99, previously mentioned in Chapters 4 and 5, respectively, and the *F. culmorum* strain F98-11 used in the original development of the leaf bioassay (Daudi, 2007).

6.2 Materials and Methods

6.2.1 The leaf bioassay

The methodology used follows that devised by Daudi (2007) with some minor modifications. The fungal isolates used were *Fusarium culmorum* isolate UK-99 (a 3-ADON and DON producer), *F. culmorum* isolate F98-11 (a 3-ADON producer) used in the original leaf bioassay development (Daudi, 2007), the sequenced *Fusarium graminearum* isolate PH-1 (a 15-ADON and DON producer) and the *tri5* mutant of *F. graminearum* PH-1 (a non 15-ADON and non DON producer) (Cuzick et al., 2008a). Inoculum was prepared by bulking conidia on SNA or PDA plates as described in chapter 2. To ensure that there

were sufficient conidia when using a *F. graminearum* isolate, a 2ml overlay of TB3 (see Chapter 2) was added and the excess removed. This was later refined to simply spreading 200 µl over the SNA culture two days prior to the experiment. Wheat leaves from two week old seedlings were selected for the assay and folded onto an elevated platform on which a cardboard strip coated in double sided tape was present. The leaves were individually attached to the cardboard with the upper leaf surface facing upwards and a single wound was made in each leaf blade using a syringe needle approximately 5-6 cm lengthways from the leaf tip, in the centre of the width of the leaf. A 7.5 µl droplet of 2.5 x 10^5 conidia/ml spore suspension in sterile distilled water was placed over the wound and the droplet held in place by the affixing of a strip of the gas permeable membrane Biofolie® 25 (Vivatech Ltd., Germany). Mock inoculations were carried out using a 7.5 µl droplet of water using the method described above. The experiment was covered with a plastic propagator lid (The Stewart Company, Croydon, UK), sealed with tape to retain high humidity and kept in place for the duration of the experiment. A black plastic bin liner was placed over the entire propagator to ensure complete darkness, and the assay remained in the dark for 24 hpi. Glasshouse conditions are described in Chapter 2. Experiments using the *F. graminearum* strains PH-1 and the *tri5* mutant were carried out in controlled environment facilities at Rothamsted Research to provide more stable conditions than the glasshouse facilities. Conditions within these facilities were set with a daily temperature regime of 23 ºC day, 18 ºC night, 16 hour day. Fluorescent lamps, type T8/HF/58W/840 (Phillips), were used with a fluence level of 300 µmol m^2 sec^{-1}.

6.2.2 Scanning Electron Microscopy

A JSM LVSEM 6360 (Jeol, UK) machine was used for this procedure, which was cooled prior to use from room temperature to -180 ºC using liquid nitrogen. Leaf segments containing leaf lesions were mounted onto cryo stubs and affixed using OCT compound and plunge frozen in liquid nitrogen. The samples were transported under vacuum to the Gatah Alto 2100 cryo chamber stage, which was maintained at -180 ºC. Fracturing, sublimation and gold

coating were done in this unit. The samples were transferred to the SEM and maintained at -150 ºC for examination. Images were recorded at various magnifications using the on board system and software. A total of eight lesions were explored using this method.

6.3 Results

6.3.1 Screening of Wheat Genotypes

To confirm the usefulness of this leaf bioassay in predicting resistance to FEB in different genotypes, the susceptible control Bobwhite and the resistant control Sumai 3 were tested in a pilot experiment and the results are given in table 6.1. This assay was inoculated with the *F. culmorum* isolate UK-99 due to the lack of conidia production on 98-11 plates, with an inoculum concentration of 6.4 x 10^5 conidia/ml.

Table 6.1 Reaction of wheat genotypes Bobwhite and Sumai 3 in the leaf bioassay

Genotype	Lesion Size (mm) Day 5	Number of Lesions Scored
Bobwhite	5.0	3
Sumai 3	2.0	3

In this experiment, Sumai 3 had a mean lesion length 3mm shorter than that of Bobwhite as expected, and a further experiment was conducted testing a wider range of genotypes to investigate their reaction in the leaf bioassay with the inoculum at a concentration of 1.26 x 10^7 conidia/ml (Table 6.2). This experiment revealed differences in lesion length between the genotypes. It should be noted than only a limited number of lesions were measured for each genotype, and as such statistical significance is not presented. Using this limited data set, some interesting trends can be seen. The susceptible control Bobwhite had a good size lesion average of 6.7mm, demonstrating that the assay was producing good lesions. The surprising result from this was that lesions of a similar size were seen on Sumai 3 leaves. Previous results from

Table 6.2 Reaction of a range of wheat genotypes in the leaf bioassay

Genotype	Inoculum	Lesion Size Day 4 (mm)	Number of Lesions Scored
Alsen	Water	0.0	1
Alsen	UK-99	11.0	3
Bobwhite	Water	0.0	1
Bobwhite	UK-99	6.7	3
China 1	Water	0.0	1
China 1	UK-99	3.3	3
China 2	Water	0.0	1
China 2	UK-99	7.0	3
China 3	Water	0.0	1
China 3	UK-99	8.3	3
China 4	Water	0.0	1
China 4	UK-99	4.7	3
China 5	Water	0.0	1
China 5	UK-99	2.7	3
China 6	Water	0.0	1
China 6	UK-99	4.0	2
China 7	Water	0.0	1
China 7	UK-99	5.3	3
China 8	Water	0.0	1
China 8	UK-99	7.3	3
Sumai 3	Water	0.0	1
Sumai 3	UK-99	6.0	2

our group suggested that lesions on Sumai 3 were no more than 1mm on average when inoculated with a *F. culmorum* isolate (Daudi, 2007). This anomalie may have been due to the increased inoculum dose in comparison to the original method by Daudi (2007), however data from a leaf bioassay using the *F. graminearum* isolate PH-1 at a concentration of 1.5 x 10^5 conidia/ml demonstrated that this was not specific to UK-99 (Table 6.3). In this experiment, standard error of the difference (SED) values are presented which allow the comparison of genotypes inoculated with the same isolate (SED genotype) or comparison of isolates on the same genotype (SED isolate). From the results of these two experiments, this assay set up was not shown to demonstrate a predictive value in identifying resistance to FEB. To investigate further the effect of isolate and the media on which the isolates were grown, some further pilot experiments were conducted. In previous experiments, when bulking conidia from *F. graminearum* isolates, a TB3 overlay was used to ensure a high concentration of conidia. Similarly, conidia from *F. culmorum* isolates were sometimes bulked on PDA plates. To investigate the effects of the different isolates and the media types, an experiment was set up, with all isolates at a concentration of 2.5 x 10^5 conidia/ml on the susceptible genotypes Bobwhite and Paragon (Table 6.4). This experiment had only small numbers of lesions for each of the different parameters, so no statistical analysis is presented. For the isolate UK-99, lesions are of a similar size regardless of the media. The results using Fg576.1 are more variable, but it is noticeable that the larger lesions are when a TB3 overlay is used. One further difference between the set up adopted in this project and that used in the previous work was the choice of glasshouse compartment. For the pilot experiment (Table 6.1) and the testing of further genotypes (Table 6.2), the leaf bioassay was carried out in the standard glasshouse (category 1, detailed in Chapter 2). The use of the *F. graminearum* isolate PH-1 (Table 6.3) necessitated the use of containment glasshouse facilities (category 2, detailed in Chapter 2). Subsequent assays were carried out in these facilities due to more consistent lesion production in the assays.

Table 6.3 Reaction of a range of wheat genotypes to the *F. graminearum* isolate PH-1

Genotype	Inoculum	Lesion Size (mm) Day 6	Number of Lesions Scored
Alsen	Water	0.0	2
Alsen	PH-1	16.6	14
Bobwhite	Water	0.0	2
Bobwhite	PH-1	9.3	14
China 1	Water	0.0	2
China 1	PH-1	10.9	14
China 2	Water	0.0	2
China 2	PH-1	13.9	14
China 3	Water	0.0	2
China 3	PH-1	16.0	14
China 4	Water	0.0	2
China 4	PH-1	9.1	14
China 5	Water	0.0	2
China 5	PH-1	9.7	12
China 6	Water	0.0	2
China 6	PH-1	8.6	10
China 7	Water	0.0	2
China 7	PH-1	8.4	14
China 8	Water	0.0	2
China 8	PH-1	10.7	13
Sumai 3	Water	0.0	2
Sumai 3	PH-1	18.3	14
SED Genotype		1.2	
SED Isolate		0.8	

Table 6.4 Effects of different isolates and media types on lesion size

Genotype	Isolate and Media Type	Lesion Length (mm) Day 7
Bobwhite	Fg576.1 SNA	3
Bobwhite	Fg576.1 SNA	17
Paragon	Fg576.1 SNA	4
Bobwhite	Fg576.1 TB3	14
Bobwhite	Fg576.1 TB3	5
Paragon	Fg576.1 TB3	20
Bobwhite	UK-99 PDA	21
Bobwhite	UK-99 PDA	20
Paragon	UK-99 PDA	22
Paragon	UK-99 PDA	24
Bobwhite	UK-99 SNA	20
Bobwhite	UK-99 SNA	30
Bobwhite	UK-99 SNA	20
Paragon	UK-99 SNA	20
Paragon	UK-99 SNA	24
Bobwhite	Water	0
Paragon	Water	0

To explore the differences between these two facilities, a further experiment was set up involving different isolates and the two media types (Table 6.5). A fresh culture of the original isolate used by Daudi (2007), F98-11, was used for this experiment as conidia could now be routinely produced *in vitro*. This experiment was done twice. The number of lesions measured for each parameter was again limited; however, some general trends could be seen. In both experiments 1 and 2, the biggest lesions were found in the category 1 facility, which was unanticipated. As mentioned previously, the category 2 facility was chosen for the assays due to more consistent lesion development. The use of TB3 for both PH-1 and Fg576.1 gave generally bigger lesions compared with the use of SNA alone. A similar effect was seen for the use of PDA plates for UK-99 in experiment 2. These results suggested that PDA plates or a TB3 overlay should not be used for the bulking of conidia. A final point from these experiments is that the use of F98-11 resulted in limited lesion development on these susceptible controls suggesting that this isolate may not be suitable for this bioassay. The next parameter to test was the effect of inoculum concentration. The initial dose suggested by Daudi (2007) was 1.5 x 10^5 conidia/ml. The use of a more dilute conidia suspension may show more differences in lesion length between the genotypes. Two concentrations of conidia were chosen: 9 x 10^4 conidia/ml and 9 x 10^3 conidia/ml, and the results are shown in table 6.6. The higher of the two concentrations was slightly lower than that suggested by Daudi (2007) due to low conidia production *in vitro* for this experiment.

Once again, the low numbers of lesions scored have meant that no statistical significance is presented. A range of lesion sizes were seen for the different genotypes examined at an inoculum concentration of 9 x 10^4 conidia/ml, but noticeably Sumai 3 has an average lesion size of 18.2 mm. Reducing the inoculum concentration resulted in reduced lesion sizes as expected, although lesion production was less consistent, with the susceptible genotype Rialto showing no lesion development at this concentration. For Sumai 3 and Bobwhite, lesions sizes were of a similar size at this reduced inoculum concentration. Due to time constraints, the set up of the leaf bioassay

Table 6.5 Effects of different isolates, media type, and glasshouse facilities on lesion size

Genotype	Isolate and Media Type	Facility	Lesion Length (mm) Day 7	
			Experiment 1	Experiment 2
Bobwhite	Fg576.1 SNA	Category 1	0	12
Bobwhite	Fg576.1 SNA	Category 1	1	15
Paragon	Fg576.1 SNA	Category 1	1	8
Paragon	Fg576.1 SNA	Category 1	-	20
Bobwhite	Fg576.1 TB3	Category 1	25	18
Bobwhite	Fg576.1 TB3	Category 1	-	20
Paragon	Fg576.1 TB3	Category 1	10	21
Paragon	Fg576.1 TB3	Category 1	-	32
Bobwhite	UK-99 PDA	Category 1	13	2
Bobwhite	UK-99 PDA	Category 1	1	0
Paragon	UK-99 PDA	Category 1	1	25
Paragon	UK-99 PDA	Category 1	-	23
Bobwhite	UK-99 SNA	Category 1	10	0
Bobwhite	UK-99 SNA	Category 1	1	0
Paragon	UK-99 SNA	Category 1	12	2
Paragon	UK-99 SNA	Category 1	1	1
Bobwhite	Water	Category 1	0	0
Paragon	Water	Category 1	0	0
Bobwhite	F98/11 PDA	Category 2	0	0
Bobwhite	F98/11 PDA	Category 2	-	0
Paragon	F98/11 PDA	Category 2	2	0
Paragon	F98/11 PDA	Category 2	2	1
Bobwhite	F98/11 SNA	Category 2	3	3
Bobwhite	F98/11 SNA	Category 2	2	4
Paragon	F98/11 SNA	Category 2	4	9
Paragon	F98/11 SNA	Category 2	2	4
Bobwhite	PH-1 SNA	Category 2	1	4
Bobwhite	PH-1 SNA	Category 2	5	2
Paragon	PH-1 SNA	Category 2	6	4
Paragon	PH-1 SNA	Category 2	2	8
Bobwhite	PH-1 TB3	Category 2	7	9
Paragon	PH-1 TB3	Category 2	12	10
Paragon	PH-1 TB3	Category 2	5	-
Bobwhite	Water	Category 2	0	0
Paragon	Water	Category 2	0	0

Table 6.6 Effect of inoculum concentration on lesion development

Genotype	Isolate	Lesion Size (mm) Day 7	Number of Lesions Scored
Bobwhite	Water	0.0	1
Bobwhite	UK-99 9×10^3 conidia/ml	1.8	4
Bobwhite	UK-99 9×10^4 condia/ml	24.6	5
China 1	Water	0.0	1
China 1	UK-99 9×10^3 conidia/ml	0.0	4
China 1	UK-99 9×10^4 condia/ml	7.4	5
CIMMYT 2	Water	0.0	1
CIMMYT 2	UK-99 9×10^3 conidia/ml	0.0	4
CIMMYT 2	UK-99 9×10^4 condia/ml	5.2	5
Consort	Water	0.0	1
Consort	UK-99 9×10^3 conidia/ml	0.0	3
Consort	UK-99 9×10^4 condia/ml	7.6	5
Paragon	Water	0.0	1
Paragon	UK-99 9×10^3 conidia/ml	0.0	4
Paragon	UK-99 9×10^4 condia/ml	11.2	5
Rialto	Water	0.0	1
Rialto	UK-99 9×10^3 conidia/ml	0.0	4
Rialto	UK-99 9×10^4 condia/ml	14.4	5
Sumai 3	Water	0.0	1
Sumai 3	UK-99 9×10^3 conidia/ml	2.0	4
Sumai 3	UK-99 9×10^4 condia/ml	18.2	5
Wuhan	Water	0.0	1
Wuhan	UK-99 9×10^3 conidia/ml	3.3	4
Wuhan	UK-99 9×10^4 condia/ml	11.2	5

to distinguish genotypes with different ear susceptibilities to FEB could not be investigated any further.

6.3.2 Investigation of infection process

Earlier experiments established that lesion development followed that seen previously by Daudi (2007). Lesions appeared within 2 days post inoculation as chlorotic spots, developing further to give elliptical lesions of approximately 10 - 20 mm lengthways (Figure 6.1). The lesion centre appeared cream in colour and in most cases spanned the width of the leaf. Lesions appeared similar regardless of the fungal species used. Lesions from Bobwhite seedlings inoculated with the isolate *F. culmorum* UK-99 were examined under the scanning electron microscope at 5 (Figure 6.3 D, E, F, H) and 7 dpi (Figure 6.3 A, B, C, G). The surfaces of the leaves were viewed as well as the interior by freeze fracturing the leaf tissue. Host cell collapse can be seen on the leaf surface when viewed from above (Figure 6.3 A). Closer inspection revealed that host stomata remained closed in healthy tissue outside of the lesion (Figure 6.3 B) and open in the necrotic lesion (Figure 6.3 C). Hyphae were frequently observed emerging from the stomatal cavity (indicated by the arrow in Figure 6.3 C). An interesting gradient was observed in the lesion boundary where the width of the leaf rapidly decreases towards the lesion centre, which mirrors the three distinct phases observed previously in the macroscopic lesions (Figure 1). This has been observed previously (Daudi, 2007). The necrotic lesion centre contains very little host cell material, with no obvious cellular structures remaining in place. Instead the resulting space was filled with a mass of fungal mycelium (Figure 6.3 D, F). Interestingly, even though the lesion centre was apparently devoid of host cells, the tips of the infected leaves still remain green (Figure 6.4), which suggested that the vascular tissue remained intact and functional. The vascular tissue was not seen under the SEM due to the angle of the sections taken. Moving further away from the lesion centre, some host cells remained intact, but others had either entirely disintegrated or were at various stages of collapse (Figure 6.3 D, G). At the outer edges of the lesion, host cells remain intact and predominantly unbranched fungal hyphae were present in the

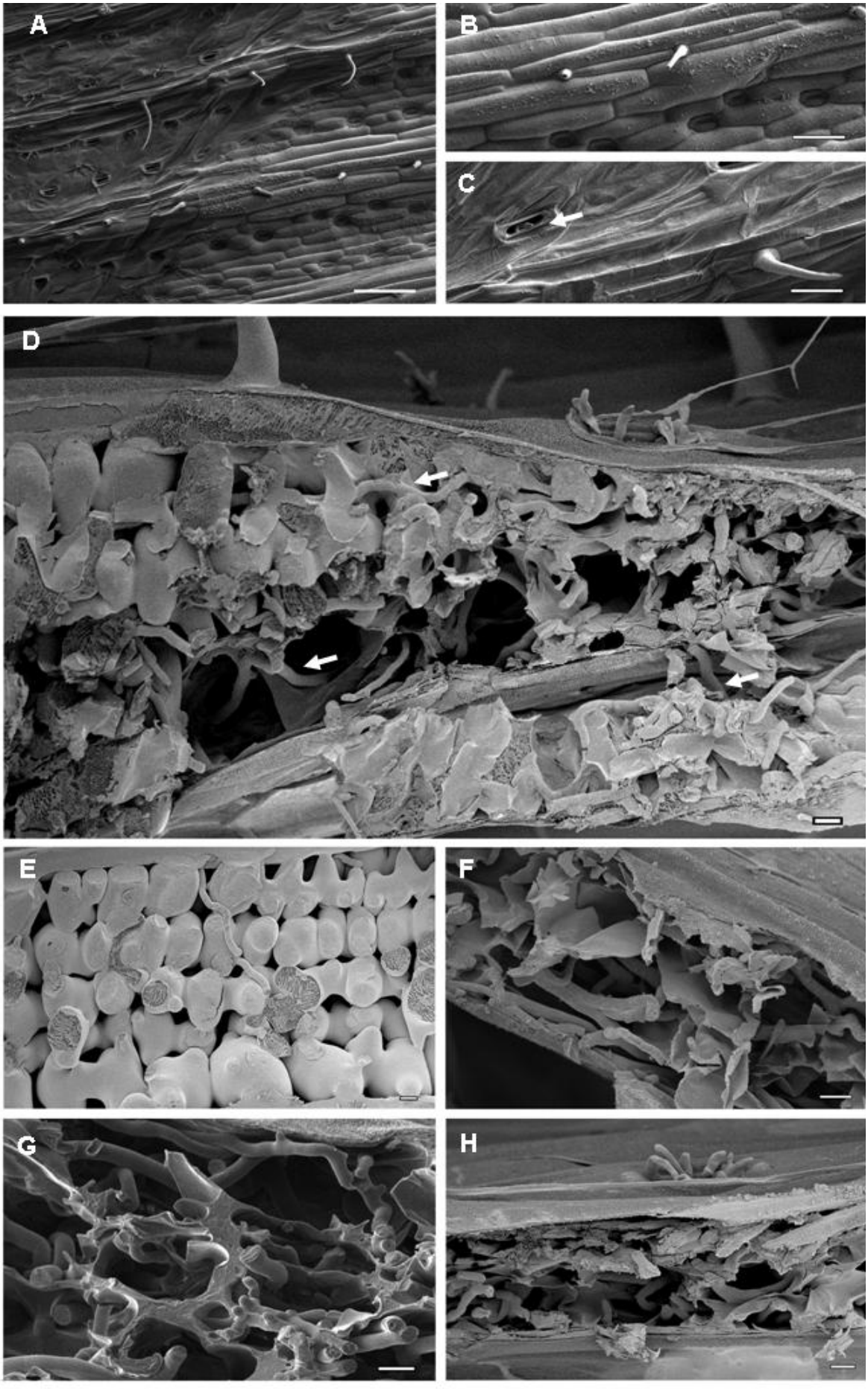

A
B
C
D
E
F
G
H

Figure 6.3 Scanning electron micrographs showing infection of wheat genotype Bobwhite leaves by *F. culmorum* using the attached leaf bioassay. A-C views of the upper leaf surface. A: Host cell collapse within the lesion is evident and a distinct boundary is seen between collapsed, infected tissue, and healthy tissue (scale bar = 200 µm). B: Cells remain turgid within the healthy tissue beyond the lesion edge and stomata remain closed (scale bar = 100 µm). C: Cells collapse within the lesion. Hyphae (arrow) have grown through open stomata onto the leaf surface. D-H views of freeze fractured lesions. D: A transect of the lesion edge. From right to left, towards the lesion centre (right hand side) hyphae are seen in vast quantities and host tissue is absent. Towards the lesion edge, the hyphae are seen throughout the tissue layers (arrows), but in lower amounts. The host tissue retains limited structure, which increases towards the healthy tissue (left hand side). At the healthier edge, hyphae are still occasionally seen in between intact cells (scale bar = 10 µm). E: Individual hyphae (arrow) have grown between healthy cells outside of the lesion ahead of the macroscopically visible symptoms (scale bar = 10 µm). F: Host tissue degraded completely in the lesion centre and entirely replaced by branched and thick mycelium. Epidermal cells remain intact but flaccid (scale bar = 10 µm). G: Within the lesion, the host cell indicated (arrow) appears to be undergoing a loss of nutrients to the surrounding hyphae (scale bar = 10 µm). H: Hyphae emerge through stomata and grow across the leaf surface (arrow). Hyphae appear flaccid in comparison to hyphae seen growing within the leaf tissue (scale bar = 10 µm).

Figure 6.4 Bobwhite seedling 7 days post inoculation with PH-1 showing green tips even though the lesion has spanned the width of the leaf.

intercellular air spaces growing over the surfaces of successive mesophyll cells as well as directly beneath the epidermal layer (Figure 6.3 D, E). Hyphae were also observed emerging from stomata at many points throughout the lesion. However, these hyphae appear flaccid in comparison to hyphae observed within the host tissue (Figure 6.3 H). Hyphae were seen up to 844 µm in advance of the collapsed host cells, although this was only carried out for two lesions (data not shown). No asexual macroconidial production was ever observed. Mock inoculated controls showed no lesion and remained green for the entire experiment.

6.3.3 Role of the mycotoxin deoxynivalenol in infection

The genotypes Bobwhite and Sumai 3 were inoculated with the wild-type *F. graminearum* isolate PH-1 and a near isogenic strain of PH-1 where the *tri5* gene had been replaced by the hygromycin selectable marker (Cuzick et al., 2008a). Lesions produced by PH-1 and the *tri5* mutant were measured at 3, 5 and 7 dpi. Results from two experiments are shown in tables 6.7 and 6.8.

Table 6.7 Lesion sizes on the genotypes Bobwhite and Sumai 3 after inoculation with PH-1 and the *tri5* mutant - rep1

		Mean Lesion Length (mm)			Number of
Genotype	Strain	Day 3	Day 5	Day 7	Lesions Scored*
Bobwhite	Water	0.0	0	0	4
	PH-1	5.4	11.12	14.75	8
	tri5	3.8	4.83	6.67	8
Sumai 3	Water	0.0	0	0	4
	PH-1	4.4	10.5	12.5	8
	tri5	4.4	8.5	10.75	8
SED Genotype		0.6	1.2	1.6	
SED Strain		0.9	1.7	2.3	

* number of lesions was reduced to 6 for each of Bobwhite PH-1, Bobwhite tri5 and Sumai 3 tri5, and 5 for Sumai 3 PH-1 on day 7 due to sampling for microscopy at day 5.

Table 6.8 Lesion sizes on the genotypes Bobwhite and Sumai 3 after inoculation with PH-1 and the *tri5* mutant - rep2

Genotype	Strain	Mean Lesion Length (mm) Day 3	Day 5	Day 7	Number of Lesions Scored
Bobwhite	Water	0.0	0.0	0.0	3
	PH-1	3.3	8.1	8.8	8
	tri5	6.9	10.3	10.8	6
Sumai 3	Water	0.0	0.0	0.0	3
	PH-1	7.4	16.5	18.6	8
	tri5	7.1	13.5	13.5	8
SED Genotype		1.0	1.2	1.3	
SED Strain		1.3	1.6	1.7	

Analysis of variance was carried out on the results and SED values are presented to allow comparison of both genotypes and inoculum types. In all experiments there were no lesions seen on mock inoculated controls. In rep 1, Bobwhite showed lesions significantly smaller for leaves inoculated with the *Tri5* mutant than the wild-type PH-1. For Sumai 3, there was no significant difference in lesion size between the strains at days 3 and 7. At day 5 the lesion sizes were significantly higher for PH-1 inoculated leaves than those inoculated with the *Tri5* mutant. In rep 2, lesions sizes are significantly higher for Bobwhite leaves inoculated with the *Tri5* mutant strain than for PH-1 inoculated leaves, in direct contrast to rep 1. For Sumai 3, there was no significant difference in lesion size between the two strains at day 3, and for days 5 and 7, the lesions were significantly smaller on leaves inoculated with the *Tri5* mutant strain than leaves inoculated with PH-1. When comparing the lesion sizes between the two genotypes, for PH-1, Sumai 3 lesions were smaller than or the same as Bobwhite lesions in rep 1. In rep 2, lesions were larger or no different on Sumai 3 leaves than Bobwhite. Closer visual inspection of the lesions revealed a difference in appearance between the two strains, with the *tri5* mutant showing a brown halo surrounding the lesion whereas the wild-type strain produced lesions with a diffuse grey border. This difference was first evident by day 4 post inoculation (Figure 6.5) and similar halos on PH-1 inoculated leaves were not noted. The brown halos were also only noted for Bobwhite leaves. The halos seen in this experiment are similar in appearance to other halos seen in previous experiments, for example when the inoculum has slipped from the leaf

Figure 6.5 *F. graminearum* PH-1 wild-type (upper) and *tri5* mutant (lower) leaf lesions on wheat susceptible cv Bobwhite (4 dpi). The lesions formed by the *tri5* mutant show a brown halo at the lesion edge.

Figure 6.6 *F. graminearum* PH-1 leaf lesion example on genotype China 1 showing a similar brown halo at the lesion edge to figure 6.5.

during set up of the assay. Halos were noted in one particular experiment carried out in the glasshouse when leaves were inoculated with the *F. graminearum* isolate PH-1 (Figure 6.6, Table 6.3). In this example, brown halos were not seen surrounding every lesion, but were seen across different genotypes. The role of these halos in the infection process of the leaf bioassay needs to be investigated further.

6.4 Discussion

The leaf bioassay was previously developed as a tool to speed up the selection of wheat genotypes for resistance to FEB (Daudi, 2007). In the original experiments, dramatic differences were seen in lesion size between Bobwhite, the susceptible control, and Sumai 3, the resistant control. One of the aims of the current study was to expand on this observation and use the leaf bioassay to distinguish resistance in further genotypes. An initial pilot experiment looked promising, with smaller lesions evident on Sumai 3 than Bobwhite. With an average of 2mm however, the lesions were a little bigger than those found by Daudi (2007). Using further genotypes, it became more apparent that lesions sizes were not always related to field resistance. Several experiments were set up to explore the reasons behind this, including facility choice, media type, and isolate. Ultimately, there was not enough time to reach a firm conclusion on the reasons behind this discrepancy and should be examined further. One of the reasons that this difference between the two studies exists may be the choice of isolates. The original study used a *F. culmorum* isolate F98-11. Unfortunately this isolate produced very low numbers of conidia *in vitro* for the majority of this study which required the use of an alternative. If this is the case, this may render the leaf bioassay unsuitable for screening material for resistance to FEB. Ear resistance to FEB is species-non-specific (Snijders and Van Eeuwijk, 1991; Van Eeuwijk et al., 1995; Bai et al., 1999; Mesterhazy et al., 2005), if lesion size was only diagnostic for resistance when a specific isolate was used; this would mean that a more isolate-specific resistance is being selected for. Another possible reason for a discrepancy is the choice of media. The original study used only SNA cultures. In this study

no firm conclusions were drawn from media type, but there was an indication that the more sugar-rich media, TB3 and PDA, may be causing larger lesions to form. This extra sugar may give the fungus a nutrient boost when colonising the leaf. Lesions were more consistently formed when the leaf bioassay was carried out in the category 2 glasshouse facility in comparison to the category 1 facility. When two facilities were directly compared, the category 1 facility appeared to produce the larger lesions which contradict the earlier conclusions. This was studied in experiments exploring multiple parameters. Investigations of these parameters in separate experiments would have been better. Due to time constraints it was not possible to do this within this project.

Scanning electron microscopy was used to explore the infection pathway of the Fusarium hyphae once established within the leaf. The previous light microscopy study (Daudi, 2007) had revealed that conidia germinate in the wound and then grew into the surrounding mesophyll cell to initiate leaf lesion formation. Inter- and intracellular hyphae were present throughout the lesion and intercellular hyphae were observed in the surrounding, apparently healthy, tissue. These results would suggest that the advancing front of hyphae was growing asymptomatically. In most cases, the lesions not only expand longitudinally, but also expand to encompass the entire width of the leaf. It was anticipated that once the green tissue across the entire leaf width had been destroyed, the upper portion of the leaf and the tip would become yellow through starvation. Interestingly, the entire distal portion of each inoculated leaf remained green throughout the duration of the experiment, suggesting that within the lesion, not all of the tissue was destroyed, and that the vascular tissue remained functional. This is in contrast to the ear infection process, where the fungus is thought to grow through the vascular bundles of the rachis (Ribichich et al., 2000) with clogging of the vascular bundle leading to premature bleaching of spikelets above the point of infection (Mesterhazy, 2002; Brennan et al., 2007). Further work is needed to prove this hypothesis. Interestingly, in the interaction between wheat leaves and the fungal pathogen *Mycosphaerella graminicola* (anamorph *Septoria tritici*) leaf lesion development

which spans the entire width of the leaf also did not result in the loss of green leaf tissue distal to the infection (Keon et al., 2007).

This leaf bioassay differs from natural infection of wheat plants because the fungus in the field does not cause lesions on the leaves, with only saprophytic growth present on plants which show diseased ears (R. Gutteridge, Rothamsted Research, pers. comm.). Macroconidia were also never produced during leaf lesion formation, unlike ear infection where macroconidia are formed to a high density, and are visible as salmon pink to red patches (Parry et al., 1995). The lack of macroconidia production also contrasts with the detached leaf bioassay developed by Diamond and Cooke (1999). Using the non-mycotoxin producing but FEB causing species, *M. majus* and *M. nivale,* their assay produced abundant sporodochia, with the leaf segments turning salmon pink during later assessments (Browne and Cooke, 2004).

To examine the influence of the DON mycotoxin on leaf lesion development, the *tri5* mutant of PH-1, the sequenced isolate of *F. graminearum* was used. DON is not essential for the initial infection of an ear, but is considered to be essential for subsequent spread of the fungus (Bai et al., 2001a) and functions as a virulence factor (Desjardins et al., 1996; Cuzick et al., 2008a). Previous investigations of this assay demonstrated that DON was produced during infection of wheat leaves (Daudi, 2007). In previously detailed leaf bioassays, DON was not a relevant factor as the fungal pathogen used, *M. nivale* and *M. majus*, do not produce mycotoxins (Hollins et al., 2003; Xu et al., 2005). The use of the isogenic wild-type and *tri5* mutant strains reveals that DON mycotoxin production is not required for leaf lesion formation by *F. graminearum*. During infection of the ear, the fungus induces a suite of cell wall degrading enzymes as well as a secreted lipase (Kang and Buchenauer, 2000b; Voigt et al., 2005). If the fungus does not rely on DON for the spread within the leaf tissue, it is possible that these enzymes are used in the colonisation of the leaf tissue. The non-requirement of DON for the leaf bioassay has important implications for the use of this assay as a selection tool. If this assay were to be used for selecting wheat genotypes for resistance to FEB in the ear, it would not be possible to select for genotypes which were resistant to DON. The type of

resistance under selection in this assay may not therefore be applicable for a practical breeding programme where resistance to DON is as important as selecting for resistance to visible disease symptoms. When the *tri5* mutant is used, leaf lesion appearance is modified and a deep brown ring becomes evident within 4 days and remains in association with the advancing edge of the expanding lesion. Spray inoculation of the *tri5* mutant strain on ears of susceptible wheat cultivar Bobwhite resulted in the production of similar eye-shaped lesions on the outer glume, encompassed by a dark ring. However, in this interaction infection was halted in the inoculated spikelets (Cuzick et al., 2008a). This contrasts with the reaction seen in the leaf bioassay, in which lesion development continued regardless of the brown ring. Evidence of brown halos in a separate experiment on the PH-1 wild-type isolate on different wheat genotypes suggest that further work is necessary to establish the mechanistic significance of the brown halo in lesion development in the leaf bioassay. In the present experiments, lesion sizes differed significantly between PH-1 and PH-1 *tri5*. The differences were significant in both replicates; however the result differed between the two. A precise role for DON and the brown ring seen in both ear and leaf lesions caused by the *tri5* mutant remain to be determined, and are the focus of a follow up histochemical investigation (Neil Brown, unpublished).

CHAPTER 7: GENERAL DISCUSSION

7.1 Summary of Findings

The principal aim of this project was to identify novel sources of resistance in hexaploid wheat to *Fusarium graminearum* and *F. culmorum*. Using the most up-to-date molecular markers available, and novel technologies, such as DArT markers, this research has proven that genotypes under investigation were not related to Sumai 3. Some of the known individual QTL known to confer FEB resistance were present in a few of the tested genotypes and may have contributed to the enhanced resistance seen under field conditions, but other genotypes lacked these QTLs and were distinct from Sumai-3. The research centred on the reaction of the eight Chinese genotypes donated to the project, and UK field trials over two years demonstrated a range of resistances. Although no genotype conferred resistance consistently as good as Sumai 3, high levels of resistance were still found in some genotypes. In combination with the marker data, this demonstrated that resistance not related to Sumai 3 has been found and this could be of use for the combining of resistance sources (discussed further in section 7.4). Further analysis of the field data with the mycotoxin levels in harvested grain demonstrated that the DON levels were lower or higher than expected in some genotypes based on macroscopic symptom development. To investigate the possible involvement of DON detoxification previously identified (Lemmens et al., 2005), eight genotypes were analysed for the presence of the conjugation product DON-3-*O*-glucoside. One of the genotypes which had the resistance locus *Fhb1* displayed such a mechanism. No other genotypes were identified apart from Sumai 3 to possess this mechanism, leading to the conclusion that other loci in the wheat genome may control this DON modification process. Alternatively, in these genotypes there could have been either a lack of DON induction or a DON degradation process activated. The DON induction possibility was explored using reporter strains to measure biomass levels, and levels of expression of the *Tri5* gene. This revealed that a limitation in the levels of fungal biomass is not the underlying cause of resistance in the genotypes

tested. However, expression of the *Tri5* gene did differ significantly, with low levels of expression evident in the genotype Alsen. Taking the results from all of the different experiments together, some interesting points begin to emerge with regard to the resistance response of the host to *Fusarium* spp. One of the main points is the discordance between visible disease symptoms and DON content of harvested grain in some genotypes. This was highlighted in the regression analysis, with the visible disease parameters explaining only 31.6 - 53.8% of the variance in the DON content. A few genotypes were identified which did not fit the hypothesis of DON and visible disease score being related. For example China 5 and China 8 contained less and more DON respectively than expected by the visible disease symptoms in the field. When this was investigated further using the *F. graminearum* reporter strains there was no significant difference between the amount of fungal biomass accumulation in these two genotypes, and the induction of *Tri5* was also not significantly different between the two genotypes. In the DON-3-*O*-glucoside experiments, the final concentration of DON was higher in China 8 than China 5 which agrees with the field results. The amount of DON-3-*O*-glucoside was also higher in China 8 than China 5, although the limitations to the experiment discussed in chapter 4 may result in a limited uptake of DON in China 5, and therefore a limited conversion to DON-3-*O*-glucoside. In the same experiment, Paragon, the susceptible control showed a low final concentration of DON and DON-3-*O*-glucoside which contrasts sharply with the field results which showed that it accumulated higher amounts of DON than expected from the visible disease symptoms. There was some good agreement however with the resistant genotypes Sumai 3 and CIMMYT 2, which both showed low levels of DON and high levels of DON-3-*O*-glucoside in the Austrian experiments, and showed low levels of DON in the UK field experiments. When CIMMYT 2 was investigated in the GUS experiments, the fungal biomass levels were not significantly lower than that of the susceptible controls, which might have been expected. Sumai 3 was unfortunately excluded from these experiments. Also surprising was that for the GUS *Tri5* induction experiments, CIMMYT 2 did not appear to have a particularly low level of induction. The Sumai 3 derivative Alsen, however, did

have a low level of *Tri5* induction which corresponded with the moderate to good levels of resistance seen in the field, and the moderate to low levels of DON in harvested grain. Conversion of DON to DON-3-*O*-glucoside was apparent in Alsen to a limited extent also. The differences in response of these genotypes highlight the complex nature of resistance to FEB. Whilst the resistance has been categorised into five types (see Chapter 1), the underlying mechanisms are still very much unknown. In the genotypes under investigation here it is possible that different mechanisms are at play to different extents making the analysis of their resistance a difficult prospect. On a practical scale, this poses a problem for the breeders trying to select wheat lines resistant to FEB. In this study, the relationship between DON and visible disease is best at the extreme ends of the scale, i.e. the resistant and the susceptible material. The genotypes which contained more or less DON than expected in harvested grain normally showed a mid-range resistance. This presents a problem for practical breeding. The costly nature of DON analysis means that screening the large numbers of wheat lines found in a commercial breeding programme is unfeasible. However from this study it is apparent that selecting genotypes with a moderate resistance can lead to an unpredictable amount of DON in tha harvested grain. The best solution for only selecting genotypes which have low DON is to therefore select for excellent FEB resistance in the field.

The leaf bioassay was also explored further by testing different wheat genotypes to compare the lesion size with resistance noted in the field. These experiments did not work out as planned due to the consistently larger lesion size found on leaves of Sumai 3. Therefore the use of this leaf bioassay as a screening tool needs to be investigated further. From a biological point of view, the infection process of the leaf bioassay was investigated using scanning electron microscopy. Fungal hyphae were found in advance of the visible lesion, and the role of DON in the formation of lesions was also investigated. A *tri5* mutant of the sequenced strain *Fusarium graminearum* PH-1 (discussed in section 7.5) was used to demonstrate that lack of the mycotoxin induces the formation of a brown halo surrounding the lesion. A similar phenotype is

observed when the identical strain is inoculated onto wheat ears (Cuzick et al., 2008a), indicating this is a conserved mechanism in different tissue types. The appearance of this halo in other experiments however suggests that this is not a phenotype solely attributable to the *tri5* mutant strain. The exact role of the halo formation is yet to be determined and is the subject of further studies within our group. Taken together, these results make a start in unravelling the route that this naturally non-leaf infecting pathogen takes during colonisation and lesion formation in the leaf bioassay.

7.2 Host Response to Infection by F. graminearum

Resistance to FEB has been extensively researched with regard to identifying genes conferring resistance which can be utilised in practical breeding programmes. The precise mechanism by which this resistance occurs has received moderate attention. The resistance response is characterised by a slower disease progression, and an early arrest when compared to a susceptible equivalent (Kang and Buchenauer, 2000a; Kang et al., 2008). Microscopically, higher numbers of hyphae are seen in the susceptible host in comparison to the resistant host (Kang and Buchenauer, 2000a). Structural defences such as appositions, papillae, and lignin deposition and the production of hydroxyprolinerich-glycoproteins occurs to a greater degree in resistant than susceptible cultivars (Kang and Buchenauer, 2000a; Siranidou et al., 2002; Kang and Buchenauer, 2003; Kang et al., 2008). The defence enzymes β-1,3-glucanase and chitinase are frequently detected in higher amounts in resistant hosts (Kang and Buchenauer, 2002; Kang et al., 2008). DON quantities are lower in resistant cultivars and have been localised to the cell walls, cytoplasm, chloroplasts and vacuoles, and associated with endoplasmic reticulum and ribosomes (Kang and Buchenauer, 2000a; Kang et al., 2008).

The profiles of metabolites produced in the host-pathogen interaction have also been explored, with differences highlighted between resistant and susceptible hosts. Metabolites such as aspartic acid, octadecanoic acid, linoleic acid, succinic acid, glyceric acid, methylmalonic acid, mannose, myo-inositol, galactose are constitutively produced in healthy wheat ears (Hamzehzarghani

et al., 2008b). Metabolites produced after inoculation include the phenolic compounds *p*-coumaric acid, *p*-cinnamic acid, ferulic acid and benzoic acid; the sugars myo-inositol, glucopyranose, melezitose; the fatty acids margaric acid and octadecanoic acid; and the organic acids butanedioic acid, isovaleric acid, methylmalonic acid, glyceric acid and malic acid (McKeehen et al., 1999; Siranidou et al., 2002; Hamzehzarghani et al., 2008b).

Gene expression analyses have also been carried out, with increased expression of the pathogenesis-related proteins PR-2, PR-3, PR-4 and PR-5 expressed at higher levels in resistant cultivars (Pritsch et al., 2000; Li et al., 2001). Genes constitutively expressed at higher levels in resistant cultivars include those for phenylpropanoid pathway enzymes, oxygen metabolism and redox enzymes, pathogenesis-related proteins (Kruger et al., 2002).

7.3 The Identification of QTL and Genes in the Wheat Genome

Resistance to FEB is inherited quantitatively, as discussed in section 1.4, chapter 1. QTL underlying quantitative traits are detected through the use of genetic markers, coupled with phenotypic data on a trait of interest in a segregating population. The segregating population is a result of a cross from two individuals which differ in the phenotype of interest (Young, 1996). Identification of QTL using this method has generated vast amounts of QTL data for many different species, but there are limitations. Errors can be introduced through the use of parents which do not differ enough in the trait of interest, resulting in poor segregation of characters. At a genetic level, this will mean that there will be fewer markers which differ between the parents and within the population, and therefore the linkage map will consist of fewer markers, i.e. it will have a low saturation. This could mean that fewer QTL are detected as some chromosome segments may be missing from the linkage map entirely, reducing the chances of finding QTL in these regions. Mapping populations which consist of too few individuals can cause misidentification of QTL, increasing the perceived contribution to the phenotype (Kelly and Vallejo, 2005). All of these errors can have costly consequences if these QTL are used in later breeding programmes.

Identification of QTL has only limited use in practical cereal breeding programmes. QTL can cover large segments of the chromosome and many genes. The inaccurate inclusion of these regions can cause problems, such as the linkage with deleterious genes on traits such as yield (reviewed by Brown, 2002). To increase the accuracy of this process, the gene(s) within a QTL need to be genetically defined. The most common method of isolating these genes in wheat involves a map-based cloning approach. Initially, only monogenic traits could be resolved using this method, however, Paterson (1988) demonstrated that QTL could be resolved as Mendelian traits, therefore being amenable to map based cloning. Following on from this, the QTL for FEB resistance on 3BS and 6B have been resolved, re-named as *Fhb1* and *Fhb2* (Cuthbert et al., 2006; Cuthbert et al., 2007). Map-based cloning in a plant species involves initial coarse mapping of a gene or QTL, narrowing the locus of interest down to an interval on a specific chromosome. Fine mapping of this interval narrows down the interval further for the initiation of chromosome walking. Large segments of the genome are cloned to form bacterial artificial chromosome (BAC) or yeast artificial chromosome (YAC) libraries. Chromosome walking utilises these libraries, either from the species of interest or from a closely related one, to ultimately give a contiguous sequence (contig) of DNA which covers the locus of interest which is then sequenced. From here, putative gene identification can take place through sequence comparison to known sequence databases. The final step in this gene cloning process is the proof of function of the putative gene (Stein and Graner, 2005). In a diploid species, with a sequenced genome, such as *Arabidopsis thaliana*, this is relatively straightforward. Isolating genes in wheat is much more challenging and this will now be discussed in further detail.

Wheat is an allohexaploid, meaning that there are three homoeologous genomes. Each of these three genomes contains homoeologous genes which can contribute differently to a phenotype. Wheat has a large genome (Table 7.1), with long stretches of repetitive DNA (Schulman et al., 2004). These stretches make it difficult to carry out chromosome walking. The life cycle of wheat is long compared to the other model plants, thus generation of mapping

populations and subsequent back-crossing lines can take many years. Until recently, the limited availability of large insert BAC libraries covering the wheat genome also hampered progress (Allouis et al., 2003). A limited number of genes have been isolated from wheat however, utilising different methods. The leaf rust resistance gene *Lr10* was cloned in 2003 (Feuillet et al., 2003). To overcome the problem of the lack of a BAC library, libraries from the diploid progenitor species of wheat (*Triticum monococcum, Triticum urartu, Aegilops speltoides,* and *Ae. tauschii*) were utilised (Stein et al., 2000). Proof of function of this gene was demonstrated by generating stable transgenic hexaploid wheat lines carrying gene over-expression constructs. A susceptible wheat genotype was transformed with the *Lr10* gene, and this resulted in resistance to leaf rust (Feuillet et al., 2003). In the same year, the *Lr21* gene was also isolated, using an *Ae. tauschii* cosmid library (Huang et al., 2003). Sequence similarity with NBS-LRR resistance genes suggested that the gene was of this class. Transformation of the susceptible wheat cultivar Fielder demonstrated a role in resistance and confirmed the gene sequence was *Lr21*. The gene was located on the short arm of chromosome 1D and covered 4359 bp (Huang et al., 2003).

The powdery mildew resistance gene *Pm3b* was cloned from hexaploid wheat using BAC libraries from *T. monococcum* (A^mA^m) and *T. durum* (AABB) (Yahiaoui et al., 2004). *Pm3b* encodes a NBS-LRR type resistance gene (Yahiaoui et al., 2004). Further alleles of this gene *Pm3a*, *Pm3d*, and *Pm3f* have been cloned (Srichumpa et al., 2005). More recently, the *Lr1* and *Yr36* genes have been cloned conferring resistance to leaf rust and stem rust, respectively (Cloutier et al., 2007; Fu et al., 2009). The *Lr1* gene was cloned using a hexaploid wheat BAC library from cv. Glenlea, which contained the *Lr1* gene (Cloutier et al., 2007). The initial locus identified contained three resistance gene analogs (RGAs), and *Lr1* segregated with one of them. Transformation of a susceptible cultivar resulted in resistance to leaf rust, and this was further confirmed using the technique of virus-induced gene silencing. *Lr1* encodes a NBS-LRR type protein. *Yr36* contrasts with the previously mentioned genes cloned, as it encodes a gene for broad spectrum resistance to a number of stripe rust races (Fu et al., 2009).

Table 7.1 Comparison of plant species genomes

Species	Diploid Number of chromosomes	Haploid Genome size (Mb)	Life cycle (weeks)	Height at maturity (m)
Triticum aestivum	42	16700	12-40+	1.0
Hordeum vulgare	14	5000	16+	1.2
Zea mays	20	2400	10+	2.0
Oryza sativa	24	441	20-30	1.2
Brachypodium distachyon	10	300	10-18	0.3
Arabidopsis thaliana	10	164	10-11	0.2

Adapted from (TAGI, 2000; Goff et al., 2002; Stein and Graner, 2005; Opanowicz et al., 2008)

Interestingly, this resistance is only evident at high temperatures (25-35 ºC) and the gene contains a kinase domain and lipid binding domain, both of which are essential for gene function. The cloning of genes from wheat still provides many problems however. The use of BAC libraries from species or genotypes other than the one of interest can be of limited use however if the gene of interest is not present in the library (Gill et al., 2004). In the case of disease resistance genes, high rates of evolution mean that this is possible (Stein and Graner, 2005). If the gene is present in these libraries, they are valuable tools, able to distinguish between the different genomes. If a BAC library of hexaploid wheat is used, two thirds of the BAC clones could be for the wrong chromosome (Stein and Graner, 2005).

In answer to some of the previously mentioned problems, the use of reference species has been proposed. The annotated genome sequences of two plant species are currently available: rice (*Orzya sativa* L. ssp. *japonica* and ssp. *indica*) and *Arabidopsis thaliana*. The publication of the sequence of *Arabidopsis thaliana* was a milestone as the first plant genome to be released (TAGI, 2000). The genome sequence of two rice sub-species followed in 2002 (Goff et al., 2002; Yu et al., 2002). The publication of these sequences were important events in plant science. The draft genome sequence of a third plant species, *Brachypodium distachyon*, is currently available (www.brachypodium.org) with a more detailed sequence expected soon (Opanowicz et al., 2008).

Comparison of the properties of different plant genomes are given in Table 7.1. *Arabidopsis* has the advantage of the smallest genome, lowest number of chromosomes and shortest life cycle making it ideal for fast molecular genetic studies. This system has provided some key insights into plant disease resistance signalling (Hammond-Kosack and Parker, 2003). The conservation of gene order between *Arabidopsis* and cereal species is limited however, and in this instance the genome sequence of rice is more applicable (Keller and Feuillet, 2000; Bennetzen and Ma, 2003). With a genome size a fraction of that of wheat, barley, and maize, rice is an ideal candidate for investigating gene function in a cereal. Comparisons made between rice and

wheat, barley, or maize provided evidence for a 'Lego' model (Moore, 1995; Moore et al., 1995). This model described how blocks of DNA are arranged in the same order on chromosomes across a range of cereal species, including rice, maize, barley, rye, and wheat. A few major differences were found, where translocations or inversions had taken place. This led to the suggestion that using blocks of rice DNA, analogous to blocks of 'Lego', the genomes of the other cereal species could be built, by using the blocks in different combinations. Subsequent differences in genome size and chromosome number were attributed to the large amounts of repetitive sequences found between genes in the larger genomes of species such as wheat (Moore et al., 1993; Schulman et al., 2004). This conservation of gene order across cereal species suggested that rice could be used as a tool for the molecular genetic analysis of the wheat genome, for example saturating a particular area with markers to aid fine mapping of a region (Keller et al., 2005). This model holds true on a macro scale, but there are deviations on a micro scale. A number of chromosomal rearrangements have been identified between wheat and rice (La Rota and Sorrells, 2004). For example, resistance gene analogs and resistance genes are not well conserved across the cereal species, suggesting that these comparative analyses are not suited to genes which evolve rapidly (Keller and Feuillet, 2000). To address this problem, a further model species has been identified: *B. distachyon*. This species has the same advantageous properties as rice, such as a small genome and gene conservation with cereal crops, in addition to other useful traits such as small stature and short generation time (Table 7.1). *B. distachyon* is more closely related to wheat and barley than rice, making it an ideal bridging species between the larger-genome cereals and rice (Opanowicz et al., 2008).

The observed colinearity between rice and wheat has also been used in the fine mapping of the *Fhb1* gene (Liu et al., 2006). Initially, the QTL on chromosome 3BS was localised to the deletion bin 0.78 - 0.87 (Liu and Anderson, 2003a), and additional markers were sought in this region by comparison to the rice genome sequence. Based on the colinearity found between wheat chromosome 3BS and rice chromosome 1S, the authors

developed further markers for the fine mapping of this area (Liu and Anderson, 2003b). Fine mapping using these additional markers demonstrated that the order of markers was different in rice and wheat. An inversion of the markers STS3B-189 and STS3B-66 in rice shows that the region containing *Fhb1* is different to that in rice, thus limiting the usefulness of rice in cloning this gene. This inversion also extended to barley.

Considering the advantages of working with model systems and the vast number of accessions and mutants already available using *Arabidopsis thaliana*, identification of potential genes involved in the host reaction to *Fusarium graminearum* and *F. culmorum* could be made simpler. The *Arabidopsis* - *Fusarium* pathosystem was developed in 2002 by Urban and co-workers (Urban et al., 2002). Spray inoculation of flowers with a conidia suspension induced disease symptoms analogous with symptoms seen on wheat ears. Flowers were frequently seen encased in fungal hyphae and the peduncle at the base of the floret was constricted in a manner similar to the colonisation of individual spikelets on wheat ears and the choking of the rachis with hyphae. DON was also produced during infection in this assay. Further work using mutants of *Arabidopsis* which were previously shown to be important in conferring disease resistance in other pathosystems demonstrated that mutants of the genes *ESA1*, *SGT1B*, *NPR1* and *EDS11* are more susceptible to infection, suggesting that these genes are involved in the resistance response in *Arabidopsis* floral tissue (Van Hemelrijck et al., 2006; Cuzick et al., 2008b; Cuzick et al., 2009). Also over expression of the Golden2-like (GLK1) transcription factor conferred resistance to fusarium floral infection in Arabidopsis (Savitch et al., 2007). Further research using a *tri5* mutant strain of *Fusarium graminearum* revealed that the production of DON is not essential for Arabidopsis infection, which contrasts with the wheat - *Fusarium* pathosystem, in which it is required for the spread of the disease (Proctor et al., 1995a; Bai et al., 2001a). In addition to these results, the transformation of susceptible cv. Bobwhite with the gene *NPR1* from *Arabidopsis* conferred increased resistance to FEB (Makandar et al., 2006) and the overexpression of the rice *NPR1* homolog, *NH1*, in the susceptible rice cultivar LiaoGeng, which gave higher

levels of resistance to *Xanthomonas oryzae* pv. *oryzae* (Chern et al., 2005), suggests that the results gained from model species experiments may have direct relevance to the generation of resistant wheat cultivars.

The final step of gene isolation is the proof of function of the gene of interest. There are many methods for achieving this, however, with its large and complex genome, there are only limited options. Transformation of wheat originated using a particle bombardment method, however, the DNA integration sites are considered to be overly complex (Jones et al., 2005). Transformation of wheat using an *Agrobacterium*-mediated system allows for a lower transgene copy number and stable integration of the transgene (Jones et al., 2005). Transformation has been used to demonstrate proof of function in most cases of gene isolation in wheat; however the disadvantages to this system are firstly the time involved in developing transgenic wheat lines, and secondly, by its nature, the generation of transgenic plants is limited in laboratories unlicensed for such activities.

Gene expression can be reduced or silenced through the use of RNA interference (RNAi) (Travella et al., 2006). Problems can arise using this method due to the polyploid nature of hexaploid wheat, which results in a degree of genetic redundancy. This means that each of the homoeologous genes present on the individual genomes must be silenced, not just a single gene. Nevertheless, gene silencing has been demonstrated in hexaploid wheat using this method for the genes *PDS* and *EIN2* (Travella et al., 2006). Silencing of the gene of interest can also be achieved by virus-induced gene silencing (VIGS). VIGS relies on the natural phenomenon of host defence against viral infection (Baulcombe, 1999). The host plant is able to reduce viral accumulation via an RNA-mediated defence system. If the virus carries the sequence or partial sequence of a host gene, this sequence will be silenced along with the virus. The VIGS system has the advantage of being faster than transformation, requiring generation of the recombinant virus (~ 2 days), and inoculation, with the silencing phenomenon establishing systemically throughout the inoculated plant within a few weeks, therefore providing a rapid method for identifying gene function (Baulcombe, 1999). The system is also better able to

deal with embryo lethal mutations. Using a standard transformation procedure, embryo lethal mutations are difficult to analyse due to the nature of the mutation and the inability to generate homozygous stable lines for analysis. (Baulcombe, 1999). Example VIGS results from studies in *Nicotiana benthamiana* have demonstrated the function of a cellulose synthase gene (Burton et al., 2000) and the roles of the genes *SGT1* and *EDS1* in defence (Peart et al., 2002a; Peart et al., 2002b). In wheat, this system has been successfully adapted and this technology is used worldwide as an alternative to stable transformation of wheat. The first published result of the VIGS system in a cereal species was using the *Barley stripe mosaic virus*, to silence the gene *PDS* which codes for the enzyme phytoene desaturase required for chlorophyll biogenesis (Holzberg et al., 2002). The silenced tissues in the plant take on a bleached appearance. In addition to this work, the VIGS system was successfully applied to wheat, and was used to demonstrate the function of the previously cloned gene *Lr21*, along with the genes *RAR1*, *SGT1*, and *HSP90*, all shown to play a part in disease resistance in other plant species (Scofield et al., 2005). Since this breakthrough, the VIGS system has been used as an alternative, or in addition, to transformation to prove gene function and VIGS was used in the demonstration of function of the recently cloned *Lr1* gene (Cloutier et al., 2007).

Mutation of the gene of interest, with the subsequent phenotypic analyses can also prove its function. Mutations can be generated using chemical mutagens, such as ethylmethyl sulphate (EMS). Another attractive option is targeting induced local lesions in genomes (TILLING) (McCallum et al., 2000). TILLING combines the use of chemical mutagens to generate a population of point mutations. These mutations are detected using denaturing high performance liquid chromatography (HPLC), which identifies mismatches in the dsDNA after the re-annealing of the strands. This system can be automated, allowing for a high throughput of samples. Further adaptations of the system include the use of *Cel I* which recognises and cleaves DNA mismatches, allowing the use of systems such as the LiCor DNA analysers used in Chapter 3. TILLING could also be used in the future for the generation of mutant variants of wheat varieties for crop improvement as an alternative to

transgenic methods (Slade et al., 2005). This was demonstrated by Slade et al (Slade et al., 2005), who identified 246 alleles of the *waxy* genes in hexaploid wheat, which ranged in phenotype from near wild type through to null. However, when confirming gene function using a TILLING approach, many independent lines harbouring mutations in the same gene need to be tested for function and a constant phenotype observed, because each line also contains hundreds of additional mutations in the genome.

Currently, there are many molecular maps available for wheat (Gupta et al., 2008), but a genome sequence of wheat would be more useful, and isolation of genes underlying important traits could be accelerated. In 2005, the International Wheat Genome Genome Sequenceing Consortium (IWGSC) (www.wheatgenome.org) was established. Recently, a physical map based on BAC clones from 'Chinese Spring' was released for the chromosome 3B by the French consortium as part of this sequencing effort (Paux et al., 2008). Chromosome 3B is considered to be the largest of the wheat chromosomes, and so could be physically isolated from the rest of the genome to facilitate cloning. This publication will likely stimulate interest in the sequencing of further chromosomes by research consortia in other countries.

7.4 Utilisation of the Information Generated by QTL Mapping

7.4.1 Generation of new wheat varieties

Information generated in the QTL mapping studies such as those discussed in chapter 1 can have practical applications in a wheat breeding programme. Wheat breeding aims to combine desirable traits such as high yield, and quality with disease and insect resistance in varieties which also demonstrate uniform development and are adapted to local climatic conditions. Combining all of these traits takes a long time and considerable investment. Current breeding programmes are focusing on problems which we may face in ten years time; such is the time for breeding a new variety (12 years). A pedigree selection method is used in most cases in which approximately 800 initial crosses generate the F_1 plants. Self-crossing of the F_1 plants yields F_2

plants which show the phenotypes of the initial cross. The best F_2 plants are screened in small rows for disease resistance, and reduce the number of F_2 plants from 2 million to 400,000. Continued screening takes place to select for good disease resistance and field characteristics such as uniformity, maturity, and lodging. After two generations screening for these properties, yield is also selected for, and is of primary concern for the end user. In addition to field qualities, end-use quality tests are also conducted, such as protein content and the Hagberg falling number. As the selection proceeds, the number of locations increases, with potential seed tested at 1, 3, and then 6 locations, before entering into National List trials and commercial evaluation. The ultimate result of this long selection process is one line. There is only a limited number of seed of this highly desirable seed, and so multiplication of the seed needs to be carried out in a highly selective manner to ensure genetic uniformity (BSPB, 2006).

This method of producing new varieties is time-consuming and laborious. Methods to reduce the time taken to select for these novel lines are currently being developed. Assays screening for disease resistance on plants at younger growth stages than required for natural resistance, such as the Fusarium leaf bioassay developed by Daudi (2007), provide quick methods for selecting sources of resistance from non-adapted germplasm or early crosses. Methods to speed up the generation time of the crosses, such as parallel breeding programmes in Northern and Southern hemispheres, generation of double haploid populations to fix the germplasm within a single generation, or single seed descent can increase the number of generations produced and tested in a year, from the standard one up to two (BSPB, 2006). Selecting for disease resistance at the seedling stage instead of adult plant stage can also greatly increase the speed of the breeding process, and is the basis of marker assisted selection (MAS).

7.4.2 Marker assisted selection

Breeding for resistance to diseases such as FEB can be complicated by the association of resistance loci to deleterious traits, such as tallness,

presence of awns, loosely arranged ears, low yield, early or late maturity, low straw strength, and susceptibility to other diseases (Somers et al., 2003). Marker assisted selection allows the breeder to screen a population for the presence of a QTL based on the presence of the markers closely associated with the QTL of interest. This approach has two main advantages. Firstly, early generations of plants can be screened at the seedling stage for known resistance QTL, negating the need to allow plants to develop to maturity before screening for resistance. Secondly, screening for markers for the deleterious genes can ensure that these genes are not present, thus breaking the linkage. This can improve the efficiency and accuracy of breeding when used in conjunction with traditional selection methods. Useful markers typically are mapped within 5 cM of the trait (Peter Jack, RAGT, Cambridge, Pers. comm.). Many QTL for FEB resistance have been identified (reviewed in section 1.4 Chapter 1), and once suitable markers have been identified, these need to be tested across different genetic backgrounds for robustness (Bai et al., 1999). Work continues to improve the existing markers, so that each marker is easy to use and as close as possible to the QTL or gene of interest (Guo et al., 2003; Liu and Anderson, 2003a, b; Yu et al., 2004). During these mapping studies, it has often been reported that transgressive segregants are present in the offspring. Transgressive segregants are offspring which display a phenotype which is more extreme than either of the parent. The best example of this for FEB resistance is Sumai 3. The parents of this particular cross were both only moderately susceptible, but gave rise to the highly resistant offspring Sumai 3 (Bai and Shaner, 1994). Therefore, by crossing plants with known sources of resistance at different loci, it should be possible to pyramid resistance from different sources and thereby create individuals with a full complement of resistance QTL in agronomically adapted varieties (Wilcoxson et al., 1992; Waldron et al., 1999; Van Sanford et al., 2001; Bai et al., 2003; McCartney et al., 2004; Wilde et al., 2008).

7.4.3 Pyramiding of resistance genes

Through the use of marker assisted selection, it is possible to select for genetic loci for resistance, not just for one disease, but for several, and for other traits, such as insect resistance. The first results are beginning to be published on the application of this technology. Somers et al. (Somers et al., 2005) provided the first evidence for pyramiding of resistance QTL. In total, six FEB QTL, resistance to orange blossom midge (*Sm1*), and leaf rust resistance (*Lr21*), were combined in different combinations. Up to four pest resistance genes or QTL were introgressed into elite cultivars in four separate crosses. Unfortunately, the authors did not describe the resistance phenotype of these genotypes. In another study (Miedaner et al., 2006) FEB QTLs were stacked in European adapted cultivars (Miedaner et al., 2006). Two QTL (3BS and 5A) came from CM82036 (Sumai 3/Thornbird) and one came from Frontana (3A). In this study, the effects of individual QTLs as well as QTL combinations were examined. The highest effect was found when the QTL on 3BS and 5A were present, reducing DON content by 78% and disease rating by 55%. Resistance QTL from European winter wheat genotypes 'Dream' and 'G16-92' have also been pyramided into elite cultivars (Wilde et al., 2008). When single QTL were introgressed, no significant difference was found in resistance, but the combination of two or three QTL lead to a significant increase in resistance.

Combination of resistance QTL from Nyuubai, Sumai 3 and Wuhan-1 in elite Canadian spring wheat cultivars showed that the 4B QTL from Wuhan-1 was most effective (McCartney et al., 2007). The resistance increased however, with pyramiding more QTL. In China, a genotype has also been developed which combines resistance QTL from Sumai 3, Wangshuibai, and Nobeokabouzu, and the authors suggested that this would provide a good resource for breeding programmes (Shi et al., 2008). Together, these results are encouraging, demonstrating that elite cultivars have the potential to be resistant to FEB. To date no one has published data which has explored the effect of combining QTLs with transgenic resistant wheat lines which exhibit good efficacy.

7.4.4 Current success utilising characterised resistance to FEB

The QTLs identified to date range in size of effect on resistance phenotype (reviewed in section 1.4, chapter 1). Ideal candidate QTL for introgression into adapted, high yielding, cultivars are ones with a high effect on the resistance phenotype. QTL choice should be approached with caution however; as QTL reported often have only been identified in one population and a limited population size can lead to the mis-identification of QTL (Utz et al., 2000). The 3BS QTL has shown to be stable over different genetic backgrounds and in some cases, the substitution of susceptible markers alleles at this locus with resistant alleles can lead to a 50% decrease in the percentage of infected spikelets (Zhou et al., 2003). The resistance from Sumai 3 has been used extensively in breeding programmes worldwide. Recently released cultivars utilising this resistance, and other well-known resistance sources include the following varieties (sources of resistance shown in brackets): 'Alsen' (Sumai 3) (Oelke and Kolmer, 2005; Frohberg et al., 2006), 'RCATL33' (Sumai 3, Frontana) (Tamburic-Ilincic et al., 2006), 'Glenn' (Sumai 3) (Mergoum et al., 2006), 'ND 756' (Sumai 3) (Mergoum et al., 2008), 'ND 751' (Sumai 3) (Mergoum et al., 2007), 'ND 744' (Sumai 3) (Mergoum et al., 2005), 'Allegiance' (unknown) (Van Sanford et al., 2006), 'Nass' (Gamenya) (Nass et al., 2006), 'Bess' (unknown) (McKendry et al., 2007), 'Granger' (Butte 86) (Glover et al., 2006), 'Ernie' (unknown) (McKendry et al., 1995) and 'Freedom' (unknown) (Gooding et al., 1997). Resistance levels are high to moderate in these cultivars and also incorporate agronomically acceptable traits such as high yield.

Cultivars 'CJ W14', 'CJ 8809', 'CJ 9306' and 'CJ 9403' were all developed through a recurrent selection programme which incorporated the resistance of not only Sumai 3, but also Ning 7840, Wangshuibai, Wenzhouhongheshang, Fanshanxiaomai, Emai 9, Zhen 7495, Jinzhou 1, Xiangmai 1, Nobeokabozu, Schinchunaga and Frontana (Jiang et al., 2006b; Jiang et al., 2006c; Jiang and Ward, 2006; Jiang et al., 2007c). It is important to obtain a wide selection of resistance mechanisms so as not to increase the

selection pressure on the pathogens to erode the effectiveness of the resistance genes involved (Yu et al., 2006).

7.5 The Availability of the Fusarium graminearum Genome

7.5.1 Classification of *F. graminearum*

To date, many species of *Fusarium* have been identified, infecting a wide range of hosts under many different climatic conditions. Within the species *F. graminearum*, at least nine different phylogenetic lineages have been identified, based on sequences of the mating type locus (MAT-1), a translation elongation factor (*EF-1 α*), a phosphate permease gene (*PHO*), β-tubulin (*TUB*), UTP-ammonia ligase (*URA*), trichothecene 3-*O*-acetyltransferase (*TRI101*) and a putative reductase (*RED*) (O'Donnell et al., 2000; O'Donnell et al., 2004). These lineages were designated as distinct species within the *Fg* clade, and two further novel species (*F. vorosii* and *F. gerlachii*) have also been described based on sequence data from 13 genes and phenotypic traits (Starkey et al., 2007). When compared with biogeographical origins of the isolates under investigation, the lineage divisions were the same, suggesting the presence of genetically different species within the *Fg* clade across the continents (O'Donnell et al., 2000). This agreement did not however extend to the trichothecene biosynthesis cluster and gene flow between the species was low (O'Donnell et al., 2000). This work has potential implications for phytosanitary control methods. Although resistance to *Fusarium* spp. is species-non-specific, introduction of a new species to an area which may be more aggressive or produce more mycotoxins could prove problematic (O'Donnell et al., 2000).

7.5.2 Sequencing of the *F. graminearum* genome

In 2003, the genome sequence of *F. graminearum* strain PH-1 was published by the Broad Institute of the Massachusetts Institute of Technology, USA (www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html). The strain PH-1 originated as a field isolate from Massachusetts and was chosen for its consistence in growth stages under laboratory conditions (Trail et al., 2005). The genome spans 36.1 Mb, spread over four chromosomes (Gale

et al., 2005). The number of predicted genes varies according to research institute. Gene number ranges from 11,640 (Broad Institute) to 14,100 (MIPS) (Cuomo et al., 2007; Trail, 2009, mips.gsf.de/genre/proj/FGDB) and currently stands at 13,332 (http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/GenomeStats.html). The discrepancy between these figures is thought to be due to the different annotation software used (Xu et al., 2006). Low levels of repetitive sequence were identified, due to a repeat-induced point mutation defence system (Cuomo et al., 2007). The sequence of a second strain of *F. graminearum*, GZ3639, was released in 2005 (Xu et al., 2006). Comparison of the sequences revealed 10,495 SNPs between the two strains (Cuomo et al., 2007). Furthermore, high rates of recombination were identified in regions containing genes expressed *in planta*, suggesting that the fungus is capable of adapting to changing environments or hosts (Cuomo et al., 2007). Following on from the publishing of the sequence, annotation of the gene functions has been carried out. Initial annotation is automated based on similarities with genes of known function. More detailed annotation is carried out manually, and eventually proof of gene function is obtained through targeted gene disruption. *Fusarium graminearum* is amenable to transformation to allow this and the current rate of manual annotation means that the *Fusarium graminearum* genome is one of the most detailed filamentous fungi genomes available (Trail et al., 2005; Trail, 2009).

7.5.3 Identification of genes involved in pathogenicity

One of the key outcomes from the genome sequencing of the pathogen is to identify genes involved in the host-pathogen interaction. Initial work in this area has focused on the identification of genes involved in the biosynthesis of mycotoxins (reviewed in chapter 1, section 1.1). Early experiments utilised the related species *F. sporotrichioides*, due to the comparative ease of production of mycotoxins under laboratory conditions. Later experiments identified the homologues in *F. graminearum*. To date, 27 genes have been identified that reduce the disease causing ability on wheat ears (excluding the *TRI* genes)

Table 7.2 Genes involved in pathogenicity in *F. graminearum*

Gene Name	Gene Product Function	Reference
ADE5	Phosphoribosylamine-glycine ligase	(Kim et al., 2007)
ARG2	Acetylglutamate synthase	(Kim et al., 2007)
FcABC1	ATP-binding cassette	(Skov et al., 2004)
CBL1	Cystathionine betalyase	(Seong et al., 2005)
CPS1	Adenylate-forming enzyme	(Lu et al., 2003)
FBP1	F-box protein	(Han et al., 2007)
FgHOG1	Mitogen activated protein kinase	(Ramamoorthy et al., 2007a)
FGL1	Secreted lipase	(Voigt et al., 2005)
FSR1	Involved in signal transduction	(Shim et al., 2006)
GCS1	Glucosylceramide synthase	(Ramamoorthy et al., 2007b)
GPMK1	Mitogen activated protein kinase	(Jenczmionka et al., 2003; Jenczmionka and Schafer, 2005)
GzGPA1, GzGPA2, GzGPA3, GzGPB1	Heterotrimeric G proteins	(Yu et al., 2008b)
GzmetE	Homoserine *O*-acetyltransferase	(Han et al., 2004)
HMR1	Hydroxymethyl-glutaryl CoA reductase	(Seong et al., 2006)
MAP1	Mitogen activated protein kinase	(Urban et al., 2003)
MGV1	Mitogen activated protein kinase	(Hou et al., 2002)
MSY1	Methionine synthase	(Seong et al., 2005)
NPS6	Nonribosomal peptide synthetase	(Oide et al., 2006)
RAS2	Ras GTPase	(Bluhm et al., 2007)
SID1	Siderophore biosynthesis	(Greenshields et al., 2007)
STE7	Mitogen activated protein kinase kinase	(Ramamoorthy et al., 2007a)
STE11	Mitogen actvated protein kinase kinase kinase	(Ramamoorthy et al., 2007a)
TBL1	Transducin beta-subunit-like gene	(Seong et al., 2005)
ZIF1	bZIP transcription factor	(Seong et al., 2005)

have been published (Table 7.2) and more than 50 are known to exist from recent conference abstracts. The genes summarised below all share the phenotype of reduced or no pathogenicity on wheat ears, but can differ markedly in other phenotypic traits. The *cbl1* and *msy1* mutants were both methionine auxotrophic (Seong et al., 2005), the *nps6* mutant was H_2O_2 sensitive (Oide et al., 2006), and the *map1* and *fsr1* mutants both had reduced perithecial formation (Urban et al., 2003; Shim et al., 2006). When some genes postulated to have a function in pathogenicity when tested reveal the mutant strains recovered displayed a wild type pathogenicity phenotype. For example, the mutants of *FgFTR1* and *FgFTR2* encoding iron permeases (Park et al., 2006). The secreted lipase gene *LIP1* demonstrated no change in pathogenicity (Feng et al., 2005), which contrasts sharply with Voigt and co-workers (Voigt et al., 2005) who found that the removal of another secreted lipase resulted in a reduced pathogenicity phenotype. This type of result may indicate some genetic redundancy in the pathogenicity factors used by *F. graminearum*. Potential pathogenicity gene targets have also been identified using proteomic analyses carried out under mycotoxin-inducing conditions which simulated conditions encountered during disease initiation (Taylor et al., 2008).

7.5.4 Development of the Affymetrix gene chip

In 2006, an Affymetrix GeneChip microarray was released based on the sequenced *F. graminearum* genome (Guldener et al., 2006b). All putative genes were included (~14,000), and the chip has potential uses for other *Fusarium* species. Data from analyses using this microarray is being collated experiment by experiment at the barleybase database (www.plexdb.org) (Guldener et al., 2006a). Analyses conducted so far include gene expression during a barley infection time course (Guldener et al., 2006b), conidial germination (Seong et al., 2008), perithecium development (Hallen et al., 2007) and during crown rot infections (Stephens et al., 2008). Gene expression in calcium ion channel mutants of *F. graminearum* (Hallen and Trail, 2008) and *Tri6* and *Tri10* mutants of *F. graminearum* (Seong et al., 2009) has also been

studied. Currently, this chip will be used to explore *F. graminearum* growing in the wheat ears of isogenic wheat lines containing or lacking the 3BS QTL (Corby Kistler, USDA, pers. comm.).

7.6 Current and Future Challenges to British Farmers

FEB is one of many diseases that affect British wheat production. Every year, cereal varieties are evaluated by the Home Grown Cereals Authority independently to the cereal breeding companies. Diseases that are tested include powdery mildew (*Blumeria graminis*), yellow rust (*Puccinia striiformis* f. sp. *tritici*), brown rust (*Puccinia triticina*), glume blotch (*Stagonospora nodorum*), septoria leaf blotch (*Mycosphaerella graminicola*), eyespot (*Oculimacula yallundae* and *Oculimacula accuformis*) and fusarium ear blight (*Fusarium* spp.). Septoria leaf blotch caused by *Mycosphaerella graminicola* is perhaps the biggest problem for UK farmers. The disease is favoured by prolonged rain in May and June which disperses pycnidia to the upper canopy leaves (Palmer and Skinner, 2002). Other problems especially in rotations where wheat is the predominant recurrent crop can include take-all, caused by *Gaemannomyces graminis* var. *tritici*, which results in "second wheat syndrome" (Bateman et al., 2008). Yields are usually reduced in the second year of continuous wheat growing, due to root damage caused by the fungus. This disease is not assessed as part of the recommended list trials due to the soil-borne, patchy nature of this disease.

Worldwide, new problems for the wheat crop may arise in the future with the emergence of a highly virulent stem rust isolate Ug99. This particular strain is capable of overcoming resistance conferred by the *Sr31* resistance gene, which along with other *Sr* genes, has conferred resistance to all known stem rust isolates in the past (Sip et al., 2005). Originating from Uganda, movement of this strain is being closely monitored, and movement of this pathogen towards India, one of the primary wheat growing areas worldwide, could cause a potential threat to food security in the future. A global rust initiative was set up by CIMMYT in the early part of this decade to tackle the problem. Monitoring of the progression of this disease and an increased effort in breeding wheat varieties for resistance will hopefully reduce the potential impact of this

devastating disease (www.cimmyt.org). In the UK, stem rust is currently not a problem because of its higher temperature requirements to cause disease.

Future disease management methods may have to take into account forthcoming EU legislation (EC directive 91/414/EEC), which restricts the use of some active ingredients. Currently, important diseases such as Septoria leaf blotch are controlled almost exclusively by azole fungicides (Cools and Fraaije, 2008), and the potential removal of some treatments could have a devastating impact on cereal production. A recent ADAS report (Clarke et al., 2008) suggested that this could result in at least a 25% reduction in productivity.

In the future, climate change is likely to play a role in determining the incidence and severity of specific plant-pathogen interactions in particular geographic regions. The most recent predictions released by the UK Climate Impacts Programme in 2002 (Hulme et al., 2002) indicate that in the 2020s the overall UK climate will warm by 1.0-1.5 ºC, and precipitation will increase by 10-20%. The winter climate is likely to be 0.5-1.0 ºC warmer, with an increase in precipitation of up to 10%, and the summers may be up to 1.5 ºC warmer, with 20% less precipitation. These changes could alter the crop species that are grown, such as an increase in maize production and other spring habit cereals. In the context of FEB, these changes could cause an increase in disease incidence. The wide-spread adoption of maize growth and conservations tilling has been linked to the increase in the incidence of FEB in America (Windels, 2000; Osborne and Stein, 2007). An increase in the temperature in the UK would make conditions more conducive for the most aggressive of the *Fusarium* species: *F. graminearum*. Currently, *F. graminearum* levels are increasing in the UK (www.cropmonitor.co.uk), and this species has previously been associated with the warmer climates of Eastern Europe and the USA (Parry et al., 1995). In addition to these changes, a drier climate may prompt the adoption of soil conservation measures, such as minimum tillage, which has been attributed to the high levels of FEB in the USA (Windels, 2000).

The influence of the changing climate on current UK cereal phytopathogens could result in some important challenges to UK farmers. *Mycosphaerella graminicola*, the causal agent of septoria leaf blotch, and

Puccinia striiformis, the causal agent of yellow rust both prefer slightly cooler conditions, and so under the climate change predictions it may be anticipated that disease levels would decrease. Recent evidence has suggested however that *Puccinia striiformis* is capable of adapting to warmer climates, as evidenced by the emergence of isolates which are highly aggressive under warm conditions in the USA (Milus et al., 2009). Further problems may also arise if the spring season becomes warmer. Currently brown rust (*Puccinia triticina*) is only seen at high levels in exceptionally warm years, such as 2004 and 2007 (www.cropmonitor.co.uk). Disease levels are normally low due to the arrival of the rust spores from continental Europe at a time when the climate is not quite warm enough to allow the disease cycle to progress. Therefore, only limited cycles of the disease take place, resulting in low levels of the disease. If the UK climate was warmer, many more cycles of the disease could take place, increasing the chances of an epidemic. Potential new threats to UK wheat production could include tan spot (*Pyrenophora tritici-repentis*) and *Barley yellow dwarf virus* (BYDV). Tan spot results in disease symptoms similar to *Mycosphaerella graminicola*, but the causal agent *P. tritici-repentis* prefers slightly warmer conditions (HGCA, 2008). Like *M. graminicola*, *P. tritici-repentis* is dispersed by rain splash. The climate predictions suggest wetter winters, which would provide ideal conditions for extensive disease spread through the autumn and winter. However, with drier summers also predicted, the importance of this disease is questionable as the most important leaves will not be present until late spring, so the disease may only cause problems for the early leaves. The other potential threat is BYDV. This virus is vectored by the bird-cherry aphid (*Rhopalosiphum padi*) and the grain aphid (*Sitobion avenae*) (HGCA, 2008). With fewer frosts predicted, combined with an EU restriction on insecticide application, the number of aphids looks set to increase, which in turn would lead to an increased potential risk of BYDV infections.

7.7 Concluding Remarks

FEB has the potential to be a big threat to wheat producers in the UK. Epidemics in the USA have seen losses of $3 billion in the 1990s, causing many farmers to lose their businesses (Windels, 2000). Control methods are

limited at the moment, relying on a more integrated approach utilising chemical and cultural control measures, and the use of resistant cultivars. Resistance to FEB is beginning to appear in adapted cultivars. One of the challenges for the breeders is to maintain high yields in addition to the incorporation of this disease resistance, which in the case of Sumai 3, comes from a source where yield is low. Ultimately, farmers will only buy seed for a high yielding variety unless there is a certainty of encountering the disease. Improving disease resistance is being made easier however through the use of marker assisted selection, which can make use of the vast quantities of QTL data being generated for this disease. Improvements to the technology through the sequencing of the wheat genome will only increase the precision by which these QTL and / or genes are incorporated. The mapping of loci involved in resistance to FEB is progressing rapidly with the identification of *Fhb1* and *Fhb2*, and the eventual isolation of the genes underlying this resistance is something to be expected in the near future.

Selection methods outside of the laboratory are also of use on a large scale where hundreds of thousands of plants are being screened. Use of assays such as the leaf bioassay can be used on a small scale to screen for resistance. Successful and sustainable resistance should be based on resistance from more than one source and based on more than one resistance mechanism. FEB is a complex problem, with fungal contamination and mycotoxin contamination providing two quite different problems to contend with. Five types of resistance have been identified previously, but in practice, only a handful of genotypes have been identified which display resistance to anything other than type II resistance to spread of the pathogen. Future work towards controlling this disease should focus on the pyramiding of resistance to kernel invasion, toxin accumulation, initial infection, as well as resistance to spread.

7.8 Further Work

Future work suggestions based on the results of this PhD could include the following:

- Further characterisation of the wheat genotypes which show different amounts of DON accumulation than expected. Genotypes which do not

accumulate as much DON could provide novel sources of resistance for pyramiding.

- Mapping of QTL underlying resistance seen in the genotypes China 1 and CIMMYT 2.
- Further exploration of fungal biomass levels and *Tri5* gene expression using the reported strains of *F. graminearum* on a wider range of wheat genotypes.
- Further investigation into the conditions necessary for the leaf bioassay to distinguish between resistant and susceptible wheat genotypes
- Investigation of the resistance mechanisms occurring in the leaf bioassay, particularly during the formation of the brown halo

REFERENCES

Abate, Z.A., Liu, S., and McKendry, A.L. (2008). Quantitative trait loci associated with deoxynivalenol content and kernel quality in the soft red winter wheat 'Ernie'. Crop Science **48,** 1408-1418.

Akbari, M., Wenzl, P., Caig, V., Carling, J., Xia, L., Yang, S., Usznski, G., Mohler, V., Lehmensiek, A., Kuchel, H., Hayden, M.J., Howes, N.K., Sharp, P., Vaughan, P., Rathmell, B., Huttner, E., and Kilian, A. (2006). Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theoretical and Applied Genetics **113,** 1409-1420.

Akhunov, E.D., Goodyear, A.W., Geng, S., Qi, L.L., Echalier, B., Gill, B.S., Miftahudin, Gustafson, J.P., Lazo, G.R., Chao, S., Anderson, O.D., Linkiewicz, A.M., Dubcovsky, J., La Rota, M., Sorrells, M.E., Zhang, D., Nguyen, H.T., Kalavacharla, V., Hossain, K., Kianian, S.F., Peng, J., Lapitan, N.L.V., Gonzalez-Hernandez, J.L., Anderson, J.A., Choi, D.W., Close, T.J., Dilbirligi, M., Gill, K.S., Walker-Simmons, M.K., Steber, C., McGuire, P.E., Qualset, C.O., and Dvorak, J. (2003). The organization and rate of evolution of wheat chromosomes are correlated with recombination rates along chromosome arms. Genome Research **13,** 753-763.

Aldred, D., and Magan, N. (2004). Prevention strategies for trichothecenes. Toxicol. Lett. **153,** 165-171.

Alexander, N.J., Hohn, T.M., and McCormick, S.P. (1998). The *TRI11* gene of *Fusarium sporotrichioides* encodes a cytochrome P-450 monooxygenase required for C-15 hydroxylation in trichothecene biosynthesis. Appl. Environ. Microbiol. **64,** 221-225.

Alexander, N.J., McCormick, S.P., and Hohn, T.M. (1999). TRI12, a trichothecene efflux pump from *Fusarium sporotrichioides*: gene isolation and expression in yeast. Mol. Gen. Genet. **261,** 977-984.

Alexander, N.J., Proctor, R.H., McCormick, S.P., and Plattner, R.D. (1997). Genetic and molecular aspects of the biosynthesis of trichothecenes by *Fusarium*. Cereal Res. Commun. **25,** 315-320.

Alexander, N.J., McCormick, S.P., Larson, T.M., and Jurgenson, J.E. (2004). Expression of *Tri15* in *Fusarium sporotrichioides*. Curr. Genet. **45,** 157-162.

Allouis, S., Moore, G., Bellec, A., Sharp, R., Rampant, F., Mortimer, K., Pateyron, S., Foote, T.N., Griffiths, S., Caboche, M., and Chalhoub, B. (2003). Construction and characterisation of a hexaploid wheat (*Triticum aestivum* L.) BAC library from the reference germplasm 'Chinese Spring'. Cereal Res. Commun. **31**.

Anderson, J.A., Stack, R.W., Liu, S., Waldron, B.L., Fjeld, A.D., Coyne, C., Moreno-Sevilla, B., Fetch, J.M., Song, Q.J., Cregan, P.B., and Frohberg, R.C. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. Theoretical and Applied Genetics **102,** 1164-1168.

Ashby, A.M., and Johnstone, K. (1993). Expression of the *E. coli* beta-glucuronidase gene in the light leaf spot pathogen *Pyrenopeziza brassicae* and its use as a reporter gene to study developmental interactions in fungi. Mycological Research **97,** 575-581.

Bai, G.H., and Shaner, G. (1994). Scab of wheat: prospects for control. Plant Disease **78,** 760-766.

Bai, G.H., and Shaner, G. (1996). Variation in *Fusarium graminearum* and cultivar resistance to wheat scab. Plant Disease **80,** 975-979.

Bai, G.H., and Shaner, G. (2004). Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. Phytopathol. **42,** 135-161.

Bai, G.H., Shaner, G., and Ohm, H. (2000). Inheritance of resistance to *Fusarium graminearum* in wheat. Theoretical and Applied Genetics **100,** 1-8.

Bai, G.H., Desjardins, A.E., and Plattner, R.D. (2001a). Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia **153,** 91-98.

Bai, G.H., Guo, P.G., and Kolb, F.L. (2003). Genetic relationships among head blight resistant cultivars of wheat assessed on the basis of molecular markers. Crop Science **43,** 498-507.

Bai, G.H., Kolb, F.L., Shaner, G., and Domier, L.L. (1999). Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. Phytopathology **89,** 343-348.

Bai, G.H., Plattner, R., Desjardins, A., and Kolb, F. (2001b). Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breeding **120,** 1-6.

Ban, T., and Suenaga, K. (2000). Genetic analysis of resistance to Fusarium head blight caused by *Fusarium graminearum* in Chinese wheat cultivar Sumai 3 and the Japanese cultivar Saikai 165. Euphytica **113,** 87-99.

Ban, T., and Watanabe, N. (2001). The effects of chromosomes 3A and 3B on resistance to Fusarium head blight in tetraploid wheat. Hereditas **135,** 95-99.

Bateman, G.L. (2005). The contribution of ground level inoculum of *Fusarium culmorum* to ear blight of winter wheat. Plant Pathology **54,** 299-307.

Bateman, G.L., Gutteridge, R.J., Jenkyn, J.F., and Self, M.M. (2008). Effects of fluquinconazole and silthiofam, applied as seed treatments to single or consecutive crops of wheat, on take-all epidemic development and grain yields. Ann. Appl. Biol. **152,** 243-254.

Bateman, G.L., Gutteridge, R.J., Gherbawy, Y., Thomsett, M.A., and Nicholson, P. (2007). Infection of stem bases and grains of winter wheat by *Fusarium culmorum* and *F. graminearum* and effects of tillage method and maize-stalk residues. Plant Pathology **56,** 604-615.

Baulcombe, D.C. (1999). Fast forward genetics based on virus-induced gene silencing. Curr. Opin. Plant Biol. **2,** 109-113.

Bechtel, D.B., Kaleikau, L.A., Gaines, R.L., and Seitz, L.M. (1985). The effects of *Fusarium graminearum* infection on wheat kernels. Cereal Chem. **62,** 191-197.

Bennett, J.W., and Klich, M. (2003). Mycotoxins. Clin. Microbiol. Rev. **16,** 497-516.

Bennetzen, J.L., and Ma, J. (2003). The genetic colinearity of rice and other cereals on the basis of genomic sequence analysis. Curr. Opin. Plant Biol. **6,** 128-133.

Birch, P.R.J., and Whisson, S.C. (2001). *Phytophthora infestans* enters the genomic era. Mol. Plant Pathol. **2,** 257-263.

Blandino, M., Minelli, L., and Reyneri, A. (2006). Strategies for the chemical control of *Fusarium* head blight: Effect on yield, alveographic parameters and deoxynivalenol contamination in winter wheat grain. Eur. J. Agron. **25,** 193-201.

Bluhm, B.H., Zhao, X., Flaherty, J.E., Xu, J.R., and Dunkle, L.D. (2007). *RAS2* regulates growth and pathogenesis in *Fusarium graminearum*. Molecular Plant Microbe Interactions **20,** 627-636.

Bottalico, A., and Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology **108,** 611-624.

Bourdoncle, W., and Ohm, H.W. (2003). Quantitative trait loci for resistance to Fusarium head blight in recombinant inbred wheat lines from the cross Huapei 57-2 / Patterson. Euphytica **131,** 131-136.

Brennan, J., Leonard, G., Cooke, B.M., and Doohan, F. (2005). Analysis of head and leaf reaction towards *Microdochium nivale*. European Journal of Plant Pathology **112,** 79-83.

Brennan, J., Fagan, B., Van Maanen, A., Cooke, B.M., and Doohan, F. (2003). Studies on *in vitro* growth and pathogenicity of European *Fusarium* fungi. European Journal of Plant Pathology **109,** 577-587.

Brennan, J.M., Leonard, G., Fagan, B., Cooke, B.M., Ritieni, A., Ferracane, R., Nicholson, P., Simpson, D., Thomsett, M., and Doohan, F. (2007). Comparison of commercial European wheat cultivars to *Fusarium* infection of head and seedling tissue. Plant Pathology **56,** 55-64.

Brown, D.W., Proctor, R.H., Dyer, R.B., and Plattner, R.D. (2003). Characterization of a *Fusarium* 2-gene cluster involved in trichothecene C-8 modification. J. Agric. Food Chem. **51,** 7936-7944.

Brown, D.W., McCormick, S.P., Alexander, N.J., Proctor, R.H., and Desjardins, A.E. (2001). A genetic and biochemical approach to study trichothecene diversity in *Fusarium sporotrichioides* and *Fusarium graminearum*. Fungal Genet. Biol. **32,** 121-133.

Brown, D.W., McCormick, S.P., Alexander, N.J., Proctor, R.H., and Desjardins, A.E. (2002). Inactivation of a cytochrome P-450 is a determinant of trichothecene diversity in *Fusarium* species. Fungal Genet. Biol. **36,** 224-233.

Brown, D.W., Dyer, R.B., McCormick, S.P., Kendra, D.F., and Plattner, R.D. (2004). Functional demarcation of the *Fusarium* core trichothecene gene cluster. Fungal Genet. Biol. **41,** 454-462.

Brown, J.K.M. (2002). Yield penalties of disease resistance in crops. Curr. Opin. Plant Biol. **5,** 1-6.

Browne, R.A. (2007). Components of resistance to fusarium head blight (FHB) in wheat detected in a seed germination assay with *Microdochium majus* and the relationship to FHB disease development and mycotoxin

accumulation from *Fusarium graminearum* infection. Plant Pathology **56,** 65-72.

Browne, R.A., and Cooke, B.M. (2004). Development and evaluation of an *in vitro* detached leaf assay for pre-screening resistance to *Fusarium* head blight in wheat. European Journal of Plant Pathology **110,** 91-102.

Browne, R.A., and Cooke, B.M. (2005). Resistance of wheat to *Fusarium* spp. in an *in vitro* seed germination assay and preliminary investigations into the relationship with *Fusarium* head blight resistance. Euphytica **141,** 23-32.

Browne, R.A., Murphy, J.P., Cooke, B.M., Devaney, D., Walsh, E.J., Griffey, C.A., Hancock, J.A., Harrison, S.A., Hart, P., Kolb, F.L., McKendry, A.L., Milus, E.A., Sneller, C., and Van Sanford, D.A. (2005). Evaluation of components of Fusarium head blight resistance in soft red winter wheat germ plasm using a detached leaf assay. Plant Disease **89,** 404-411.

Bruins, M.B.M., Karsai, I., Schepers, J., and Snijders, C.H.A. (1993). Phytotoxicity of deoxynivalenol to wheat tissue with regard to *in vitro* selection for Fusarium head blight resistance. Plant Sci. **94,** 195-206.

BSPB. (2006). Plant Breeding The business and science of crop improvement. (Ely, UK: BSPB).

Buerstmayr, H., Lemmens, M., Berlakovich, S., and Ruckenbauer, P. (1999a). Combining ability of resistance to head blight caused by *Fusarium culmorum* (W.G. Smith) in the F_1 of a seven parent diallel of winter wheat (*Triticum aestivum* L.). Euphytica **110,** 199-206.

Buerstmayr, H., Lemmens, M., Fedak, G., and Ruckenbauer, P. (1999b). Back-cross reciprocal monosomic analysis of Fusarium head blight resistance in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics **98,** 76-85.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., and Ruckenbauer, P. (2002). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theoretical and Applied Genetics **104,** 84-91.

Buerstmayr, H., Steiner, B., Hartl, L., Griesser, M., Angerer, N., Lengauer, D., Miedaner, T., Schneider, B., and Lemmens, M. (2003). Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. II. Resistance to fungal penetration and spread. Theoretical and Applied Genetics **107,** 503-508.

Bunkers, G.J. (1991). Expression of the *Escherichia coli* beta-glucuronidase gene in *Pseudocercosporella herpotrichoides*. Appl. Environ. Microbiol. **57,** 2896-2900

Burgess, L.W. (1981). General ecology of the Fusaria. In Fusarium: Diseases, Biology, and Taxonomy, P.E. Nelson, T.A. Toussoun, and R.J. Cook, eds (University Park and London: The Pennsylvania State University Press), pp. 225-235.

Burton, R.A., Gibeaut, D.M., Bacic, A., Findlay, K., Roberts, K., Hamilton, A., Baulcombe, D.C., and Fincher, G.B. (2000). Virus-induced silencing of a plant cellulose synthase gene. The Plant Cell **12,** 691-705.

Carris, L.M., Castlebury, L.A., and Goates, B.J. (2006). Nonsystemic bunt fungi - *Tilletia indica* and *T. horrida*: A review of history, systematics, and biology. Annu. Rev. Phytopathol. **44,** 113-133.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. (1994). Green fluorescent protein as a marker for gene expression. Science **263,** 802-805.

Champeil, A., Dore, T., and Fourbet, J.F. (2004). Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by *Fusarium* in wheat grains. Plant Sci. **166,** 1389-1415.

Chen, J., Griffey, C.A., Saghai Maroof, M.A., Stromberg, E.L., Biyashev, R.M., Zhao, W., Chappell, M.R., Pridgen, T.H., Dong, Y., and Zeng, Z. (2006). Validation of two major quantitative trait loci for Fusarium head blight resistance in Chinese wheat line W14. Plant Breeding **125,** 99-101.

Chen, L.F., McCormick, S.P., and Hohn, T.M. (2000). Altered regulation of 15-acetyldeoxynivalenol production in *Fusarium graminearum*. Appl. Environ. Microbiol. **66,** 2062-2065.

Chern, M., Fitzgerald, H.A., Canlas, P.E., Navarre, D.A., and Ronald, P.C. (2005). Overexpression of a rice NPR1 homolog leads to constitutive activation of defense response and hypersensitivity to light. Molecular Plant Microbe Interactions **18,** 511-520.

Chisholm, S.T., Coaker, G., Day, B., and Staskawicz, B.J. (2006). Host-microbe interactions: Shaping the evolution of the plant immune response. Cell **124,** 803-814.

Clarke, J., Gladders, P., Green, K., Lole, M., Ritchie, F., Twining, S., and Wynn, S. (2008). Evaluation of the impact on UK agriculture for the proposal for a regulation of the European Parliament and of the council concerning the placing of plant protection products on the market (Boxworth: ADAS).

Cloutier, S., McCallum, B.D., Loutre, C., Banks, K., Wicker, T., Feuillet, C., Keller, B., and Jordan, M.C. (2007). Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. Plant Mol.Biol. **65,** 93-106.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., and Pang, E.C.K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica **142,** 169-196.

Comai, L., Young, K., Till, B.J., Reynolds, S.H., Greene, E.A., Codomo, C.A., Enns, L.C., Johnson, J.E., Burtner, C., Odden, A.R., and Henikoff, S. (2004). Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. Plant J. **37,** 778-786.

Cools, H.J., and Fraaije, B.A. (2008). Are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in *Mycosphaerella graminicola*. Pest Management Science **64,** 681-684.

Couteaudier, Y., Daboussi, M.J., Eparvier, A., Langin, T., and Orcival, J. (1993). The GUS gene fusion system (*Escherichia coli* beta-D-glucuronidase gene), a useful tool in studies of root colonization by *Fusarium oxysporum*. Appl. Environ. Microbiol. **59,** 1767-1773.

Cuomo, C.A., Guldener, U., Xu, J.R., Trail, F., Turgeon, B.G., Di Pietro, A., Walton, J.D., Ma, L.J., Baker, S.E., Rep, M., Adam, G., Antoniw, J., Baldwin, T., Calvo, S., Chang, Y.L., DeCaprio, D., Gale, L.R., Gnerre, S., Goswami, R.S., Hammond-Kosack, K., Harris, L.J., Hilburn, K., Kennell, J.C., Kroken, S., Magnuson, J.K., Mannhaupt, G., Mauceli, E., Mewes, H.W., Mitterbauer, R., Muehlbauer, G., Munsterkotter, M., Nelson, D., O'Donnell, K., Ouellet, T., Qi, W.H., Quesneville, H., Roncero, M.I.G., Seong, K.Y., Tetko, I.V., Urban, M., Waalwijk, C., Ward, T.J., Yao, J.Q., Birren, B.W., and Kistler, H.C. (2007). The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science **317,** 1400-1402.

Cuthbert, P.A., Somers, D.J., and Brule-Babel, A. (2007). Mapping of *Fhb2* on chromosome 6BS: a gene controlling Fusarium head blight field resistance in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics **114,** 429-437.

Cuthbert, P.A., Somers, D.J., Thomas, J., Cloutier, S., and Brule-Babel, A. (2006). Fine mapping *Fhb1*, a major gene controlling fusarium head blight resistance in bread wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics **112,** 1465-1472.

Cuzick, A., Urban, M., and Hammond-Kosack, K. (2008a). *Fusarium graminearum* gene deletion mutants *map1* and *tri5* reveal similarities and differences in the pathogenicity requirements to cause disease on Arabidopsis and wheat floral tissue. New Phytol. **177,** 990-1000.

Cuzick, A., Maguire, K., and Hammond-Kosack, K.E. (2009). Lack of the plant signalling component SGT1b enhances disease resistance to *Fusarium culmorum* in Arabidopsis buds and flowers. New Phytol. **181,** 901-912.

Cuzick, A., Lee, S., Gezan, S., and Hammond-Kosack, K. (2008b). NPR1 and EDS11 contribute to host resistance against *Fusarium culmorum* in Arabidopsis buds and flowers. Mol. Plant Pathol. **9,** 697-704.

Daudi, A. (2007). *An exploration of* Fusarium *mycotoxin accumulation in hexaploid wheat germplasm*. PhD Thesis, University of East Anglia, Norwich

Dawson, W.A.J.M., Jestoi, M., Rizzo, A., Nicholson, P., and Bateman, G.L. (2004). Field evaluation of fungal competitors of *Fusarium culmorum* and *F. graminearum,* causal agents of ear blight of winter wheat, for the control of mycotoxin production in grain. Biocontrol Sci. Technol. **14,** 783-799.

de la Pena, R.C., and Murray, T.D. (1994). Identifying wheat genotypes resistant to eyespot disease with a beta-glucuronidase-transformed strain of *Pseudocercosporella herpotrichoides*. Phytopathology **84,** 972-976.

Demeke, T., Clear, R.M., Patrick, S.K., and Gaba, D. (2005). Species-specific PCR-based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. International Journal of Food Microbiology **103,** 271-284.

Desjardins, A.E., Hohn, T.M., and McCormick, S.P. (1993). Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. Microbiol. Rev. **57,** 595-604.

Desjardins, A.E., Proctor, R.H., Bai, G.H., McCormick, S.P., Shaner, G., Buechley, G., and Hohn, T.M. (1996). Reduced virulence of trichothecene-nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant-Microbe Interact. **9,** 775-781.

Diamond, H., and Cooke, B.M. (1999). Towards the development of a novel *in vitro* strategy for early screening of *Fusarium* ear blight resistance in adult winter wheat plants. European Journal of Plant Pathology **105,** 363-372.

Diamond, H., and Cooke, B.M. (2003). Preliminary studies on biological control of the Fusarium ear blight complex of wheat. Crop Prot. **22,** 99-107.

Doohan, F.M., Smith, P., Parry, D.W., and Nicholson, P. (1998). Transformation of *Fusarium culmorum* with the beta-D-glucuronidase (GUS) reporter gene: a system for studying host-pathogen relationships and disease control. Physiol. Mol. Plant Pathol. **53,** 253-268.

Doohan, F.M., Weston, G., Rezanoor, H.N., Parry, D.W., and Nicholson, P. (1999). Development and use of a reverse transcription-PCR assay to study expression of *Tri5* by *Fusarium* species in vitro and in planta. Appl. Environ. Microbiol. **65,** 3850-3854.

Draeger, R., Gosman, N., Steed, A., Chandler, E., Thomsett, M., Srinivasachary, Schondelmaier, J., Buerstmayr, H., Lemmens, M., Schmolke, M., Mesterhazy, A., and Nicholson, P. (2007). Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theoretical and Applied Genetics **115,** 617-625.

Dyer, R.B., Kendra, D.F., and Brown, D.W. (2006). Real-time PCR assay to quantify *Fusarium graminearum* wild-type and recombinant mutant DNA in plant material. J. Microbiol. Methods **67,** 534-542.

Dyer, R.B., Plattner, R.D., Kendra, D.F., and Brown, D.W. (2005). *Fusarium graminearum TRI14* is required for high virulence and DON production on wheat but not for DON synthesis in vitro. J. Agric. Food Chem. **53,** 9281-9287.

Edwards, S.G., Pirgozliev, S.R., Hare, M.C., and Jenkinson, P. (2001). Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat. Appl. Environ. Microbiol. **67,** 1575-1580.

Ellis, M.H., Spielmeyer, W., Gale, K.R., Rebetzke, G.J., and Richards, R.A. (2002). "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theoretical and Applied Genetics **105,** 1038-1042.

Evans, C.K., Xie, W., Dill-Macky, R., and Mirocha, C.J. (2000). Biosynthesis of deoxynivalenol in spikelets of barley inoculated with macroconidia of *Fusarium graminearum*. Plant Disease **84,** 654-660.

Feinberg, B., and McLaughlin, C.S. (1989). Biochemical mechanism of action of trichothecene mycotoxins. In Trichothecene mycotoxicosis:

Pathophysiologic effects, V.R. Beasly, ed (Boca Raton: CRC Press), pp. 27-33.

Feng, J., Liu, G.S., Selvaraj, G., Hughes, G.R., and Wei, Y.D. (2005). A secreted lipase encoded by *LIP1* is necessary for efficient use of saturated triglyceride lipids in *Fusarium graminearum*. Microbiology **151,** 3911-3921.

Fernandez, M.R., and Chen, Y. (2005). Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. Plant Disease **89,** 164-169.

Fernando, W.G.D., Paulitz, T.C., Seaman, W.L., Dutilleul, P., and Miller, J.D. (1997). Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. Phytopathology **87,** 414-421.

Feuillet, C., and Keller, B. (2004). Molecular markers for disease resistance: the example wheat. In Biotechnology in Agriculture and Forestry, H. Lorz and G. Wenzel, eds (Berlin: Springer), pp. 355-370.

Feuillet, C., Travella, S., Stein, N., Albar, L., Nubalt, A., and Keller, B. (2003). Map-based isolation of the leaf rust disease resistance gene *Lr10* fromthe hexaploid wheat (*Triticum aestivum* L.) genome. Proc. Natl. Acad. Sci. U. S. A. **100,** 15253-15258.

Flor, H.H. (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. **9,** 275-296.

Fredlund, E., Gidlund, A., Olsen, M., Borjesson, T., Sphid, N.H.H., and Simonsson, M. (2008). Method evaluation of *Fusarium* DNA extraction from mycelia and wheat for down-stream real-time PCR quantification and correlation to mycotoxin levels. J. Microbiol. Methods **73,** 33-40.

Frohberg, R.C., Stack, R.W., Olson, T., Miller, J.D., and Mergoum, M. (2006). Registration of 'Alsen' wheat. Crop Science **46,** 2311-2312.

Fu, D., Uauy, C., Distelfield, A., Blechl, A., Epstein, L., Chen, X., Sela, H., Fahima, T., and Dubcovsky, J. (2009). A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. Science **323,** 1357-1360.

Gale, L.R., Bryant, J.D., Calvo, S., Giese, H., Katan, T., O'Donnell, K., Suga, H., Taga, M., Usgaard, T.R., Ward, T.J., and Kistler, H.C. (2005). Chromosome complement of the fungal plant pathogen *Fusarium graminearum* based on genetic and physical mapping and cytological observations. Genetics **171,** 985-1001.

Gervais, L., Dedryver, F., Morlais, J.Y., Bodusseau, V., Negre, S., Bilous, M., Groos, C., and Trottet, M. (2003). Mapping of quantitative trait loci for field resistance to Fusarium head blight in an European winter wheat. Theoretical and Applied Genetics **106,** 961-970.

Gilbert, J., and Tekauz, A. (2000). Review: Recent developments in research on fusarium head blight of wheat in Canada. Canadian Journal of Plant Pathology **22,** 1-8.

Gilbert, J., and Fernando, W.G.D. (2004). Epidemiology and biological control of Gibberella zeae / Fusarium graminearum. Canadian Journal of Plant Pathology **26,** 464-472.

Gill, B.S., Appels, R., Botha-Oberholster, A.M., Buell, C.R., Bennetzen, J.L., Chalhoub, B., Chumley, F., Dvorak, J., Iwanaga, M., Keller, B., Li, W.,

McCombie, W.R., Ogihara, Y., Quetier, F., and Sasaki, T. (2004). A workshop report on wheat genome sequencing. Genetics **168,** 1087-1096.

Gilsinger, J., Kong, L., Shen, X., and Ohm, H. (2005). DNA markers associated with low Fusarium head blight incidence and narrow flower opening in wheat. Theoretical and Applied Genetics **110,** 1218-1225.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. **43,** 205-227.

Glover, K.D., Rudd, J.C., Devkota, R.N., Hall, R.G., Jin, Y., and Rickertsen, J.R. (2006). Registration of 'Granger' wheat. Crop Science **46,** 1390-1391.

Glynn, N.C. (2005). Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties *majus* and *nivale* to species status. Mycological Research **109,** 872-880.

Glynn, N.C., Ray, R., Edwards, S.G., Hare, M.C., Parry, D.W., Barnett, C.J., and Beck, J.J. (2007). Quantitative *Fusarium* spp. and *Microdochium* spp. PCR assays to evaluate seed treatments for the control of *Fusarium* seedling blight of wheat. J. Appl. Microbiol. **102,** 1645-1653.

Goff, S.A., Ricke, D., Lan, T.H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Varma, H., Hadley, D., Hutchinson, D., Martin, C., Katagiri, F., Lange, B.M., Moughamer, T., Xia, Y., Budworth, P., Zhong, J., Miguel, T., Paszkowski, U., Zhang, S., Colbert, M., Sun, W.L., Chen, L., Cooper, B., Park, S., Wood, T.C., Mao, L., Quail, P., Wing, R., Dean, R., Yu, Y., Zharkikh, A., Shen, R., Sahasrabudhe, S., Thomas, A., Cannings, R., Gutin, A., Pruss, D., reid, J., Tavtigian, S., Mitchell, J., Eldredge, G., Scholl, T., Miller, R.M., Bhatnagar, S., Adey, N., Rubano, T., Tusneem, N., Robinson, R., Feldhaus, J., Macalma, T., Oliphant, A., and Briggs, S. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science **296,** 92-100.

Gooding, R.W., Lafever, H.N., Campbell, K.G., and Herald, L.D. (1997). Registration of 'Freedom' wheat. Crop Science **37,** 1007.

Gosman, N., Bayles, R., Jennings, P., Kirby, J., and Nicholson, P. (2007). Evaluation and characterization of resistance to fusarium head blight caused by *Fusarium culmorum* in UK winter wheat cultivars. Plant Pathology **56,** 264-276.

Goswami, R.S., and Kistler, H.C. (2004). Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. **5,** 515-525.

Grausgruber, H., Lemmens, M., Burstmayr, H., and Ruckenbauer, P. (1999). Resistance of 'Chinese Spring' substitution lines carrying chromosomes from 'Cheyenne', 'Hope' and 'Lutescens 62' wheats to head blight caused by *Fusarium culmorum*. Hereditas **130,** 57-63.

Greenshields, D.L., Liu, G., Feng, J., Selvaraj, G., and Wei, Y. (2007). The siderophore biosynthetic gene *SID1*, but not the ferroxidase gene *FET3*, is required for full *Fusarium graminearum* virulence. Mol. Plant Pathol. **8,** 411-421.

Guldener, U., Mannhaupt, G., Munsterkotter, M., Haase, D., Oesterheld, M., Stumpflen, V., Mewes, H.-W., and Adam, G. (2006a). FGDB: a

comprehensive fungal genome resource on the plant pathogen *Fusarium graminearum*. Nucleic Acids Research **34,** D456-D458.

Guldener, U., Seong, K.Y., Boddu, J., Cho, S.H., Trail, F., Xu, J.R., Adam, G., Mewes, H.W., Muehlbauer, G.J., and Kistler, H.C. (2006b). Development of a *Fusarium graminearum* Affymetrix GeneChip for profiling fungal gene expression in vitro and in planta. Fungal Genet. Biol. **43,** 316-325.

Guo, P.G., Bai, G.H., and Shaner, G.E. (2003). AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat. Theoretical and Applied Genetics **106,** 1011-1017.

Gupta, P.K., Mir, R.R., Mohan, A., and Kumar, J. (2008). Wheat genomics: present status and future prospects. International Journal of Plant Genomics **2008,** 1-36.

Haberle, J., Schweizer, G., Schondelmaier, J., Zimmermann, G., and Hartl, L. (2009). Mapping QTL for resistance against *Fusarium* head blight in the winter wheat population Pelikan//Bussard/Ning8026. Plant Breeding **128,** 27-35.

Haberle, J., Schmolke, M., Schweizer, G., Korzun, V., Ebmeyer, E., Zimmermann, G., and Hartl, L. (2007). Effects of two major fusarium head blight resistance QTL verified in a winter wheat backcross population. Crop Science **47,** 1823-1831.

Hallen, H.E., and Trail, F. (2008). The L-type calcium growth ion channel Cch1 affects ascospore discharge and mycelial growth in the filamentous fungus *Gibberella zeae* (Anamorph *Fusarium graminearum*) Eukaryotic Cell **7,** 415-424.

Hallen, H.E., Huebner, M., Shiu, S.H., Guldener, U., and Trail, F. (2007). Gene expression shifts during perithecium development in *Gibberella zeae* (anamorph *Fusarium graminearum*), with particular emphasis on ion transport proteins. Fungal Genet. Biol. **44,** 1146-1156.

Hammond-Kosack, K.E., and Parker, J.E. (2003). Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. Curr. Opin. Biotechnol. **14,** 177-193.

Hammond-Kosack, K.E., and Kanyuka, K. (2007). Resistance genes (*R* genes) in plants. In Encyclopedia of Life Sciences (Chichester, UK: John Wiley & Sons), pp. 1-21.

Hamzehzarghani, H., Paranidharan, V., Abu-Nada, Y., Kushalappa, A.C., Mamer, O., and Somers, D. (2008a). Metabolic profiling to discriminate wheat near isogenic lines, with quantitative trait loci at chromosome 2DL, varying in resistance to fusarium head blight. Can. J. Plant Sci. **88,** 789-797.

Hamzehzarghani, H., Paranidharan, V., Abu-Nada, Y., Kushalappa, A.C., Dion, Y., Rioux, S., Comeau, A., Yaylayan, V., and Marshall, W.D. (2008b). Metabolite profiling coupled with statistical analyses for potential high-throughput screening of quantitative resistance to fusarium head blight in wheat. Canadian Journal of Plant Pathology **30,** 24-36.

Han, Y.K., Lee, T., Han, K.H., Yun, S.H., and Lee, Y.W. (2004). Functional analysis of the homoserine *O*-acetyltransferase gene and its

identification as a selectable marker in *Gibberella zeae*. Curr. Genet. **46,** 205-212.

Han, Y.K., Kim, M.D., Lee, S.H., Yun, S.H., and Lee, Y.W. (2007). A novel F-box protein involved in sexual development and pathogenesis in *Giberella zeae*. Mol. Microbiol. **63,** 768-779.

Hartl, D.L., and Jones, E.W. (1999). Essential Genetics. (Sudbury: Jones and Bartlett Publishers).

HGCA. (2008). The encyclopaedia of cereal diseases. (HGCA, BASF).

Hilton, A.J., Jenkinson, P., Hollins, T.W., and Parry, D.W. (1999). Relationship between cultivar height and severity of Fusarium ear blight in wheat. Plant Pathology **48,** 202-208.

Hohn, T.M., and Vanmiddlesworth, F. (1986). Purification and characterization of the sequiterpene cyclase trichodiene synthetase from *Fusarium sporotrichioides*. Archives of Biochemistry and Biophysics **251,** 756-761.

Hohn, T.M., and Beremand, P.D. (1989). Isolation and nucleotide sequence of a sesquiterpene cyclase gene from the trichothecene-producing fungus *Fusarium sporotrichioides*. Gene **79,** 131-138.

Hohn, T.M., Desjardins, A.E., and McCormick, S.P. (1995). The *Tri4* gene of *Fusarium sporotrichioides* encodes a cytochrome P450 monooxygenase invloved in trichothecene biosynthesis. Mol. Gen. Genet. **248,** 95-102.

Hohn, T.M., Krishna, R., and Proctor, R.H. (1999). Characterization of a transcriptional activator controlling trichothecene toxin biosynthesis. Fungal Genet. Biol. **26,** 224-235.

Hollins, T.W., Ruckenbauer, P., and De Jong, H. (2003). Progress towards wheat varieties with resistance to Fusarium head blight. Food Control **14,** 239-244.

Holzapfel, J., Voss, H.-H., Miedaner, T., Korzun, V., Haberle, J., Schweizer, G., Mohler, V., Zimmermann, G., and Hartl, L. (2008). Inheritance of resistance to Fusarium head blight in three European winter wheat populations. Theoretical and Applied Genetics **117,** 1119-1128.

Holzberg, S., Brosio, P., Gross, C., and Pogue, G.P. (2002). *Barley stripe mosaic virus*-induced gene silencing in a monocot plant. The Plant Journal **30,** 315-327.

Homdork, S., Fehrmann, H., and Beck, R. (2000). Effects of field application of tebuconazole on yield, yield components and the mycotoxin content of *Fusarium*-infected wheat grain. J. Phytopathol. **148,** 1-6.

Hooker, D.C., Schaafsma, A.W., and Tamburic-Ilincic, L. (2002). Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat. Plant Disease **86,** 611-619.

Hope, R., and Magan, N. (2003). Two-dimensional environmental profiles of growth, deoxynivalenol and nivalenol production by Fusarium culmorum on a wheat-based substrate. Lett. Appl. Microbiol. **37,** 70-74.

Hope, R., Aldred, D., and Magan, N. (2005). Comparison of environmental profiles for growth and deoxynivalenol production by Fusarium culmorum and F. graminearum on wheat grain. Lett. Appl. Microbiol. **40,** 295-300.

Hou, Z.M., Xue, C.Y., Peng, Y.L., Katan, T., Kistler, H.C., and Xu, J.R. (2002). A mitogen-activated protein kinase gene (*MGV1*) in *Fusarium*

graminearum is required for female fertility, heterokaryon formation, and plant infection. Mol. Plant-Microbe Interact. **15,** 1119-1127.

Huang, L., Brooks, S.A., Li, W., Fellers, J.P., Trick, H.N., and Gill, B.S. (2003). Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics **164,** 655-664.

Hulme, M., Turnpenny, J., and Jenkins, G. (2002). Climate change scenarios for the United Kingdom: The UKCIP02 briefing report (Norwich, UK: Tyndall Centre for Climate Change Research, School of Environmental Sciences, University of East Anglia), pp. 15.

Ichinoe, M., Kurata, H., Sugiura, Y., and Ueno, Y. (1983). Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol. **46,** 1364-1369.

Inch, S.A., and Gilbert, J. (2003). Survival of *Gibberella zeae* in *Fusarium*-damaged wheat kernels. Plant Disease **87,** 282-287.

Jaccoud, D., Peng, K., Feinstein, D., and Kilian, A. (2001). Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Research **29,** e25.

Jackowiak, H., Packa, D., Wiwart, M., and Perkowski, J. (2005). Scanning electron microscopy of Fusarium damaged kernels of spring wheat. International Journal of Food Microbiology **98,** 113-123.

Jansen, C., von Wettstein, D., Schafer, W., Kogel, K.-H., Felk, A., and Maier, F.J. (2005). Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum*. Proc. Natl. Acad. Sci. U. S. A. **102,** 16892-16897.

Jefferson, R.A., Burgess, S.M., and Hirsh, D. (1986). Beta-glucuronidase from *Escherichia coli* as a gene-fusion marker. Proc. Natl. Acad. Sci. U. S. A. **83,** 8447-8451.

Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W. (1987). GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO Journal **6,** 3901-3907.

Jenczmionka, N.J., and Schafer, W. (2005). The Gpmk1 MAP kinase of *Fusarium graminearum* regulates the induction of specific secreted enzymes. Curr. Genet. **47,** 29-36.

Jenczmionka, N.J., Maier, F.J., Losch, A.P., and Schafer, W. (2003). Mating, conidiation and pathogenicity of *Fusarium graminearum*, the main causal agent of the head blight disease of wheat, are regulated by the MAP kinase gpmk1. Curr. Genet. **43,** 87-95.

Jenkinson, P., and Parry, D.W. (1994). Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. Mycological Research **98,** 506-510.

Jennings, P., Coates, M.E., Turner, J.A., Chandler, E.A., and Nicholson, P. (2004). Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. Plant Pathology **53,** 182-190.

Jia, G.F., Chen, P.D., Qin, G.J., Bai, G.H., Wang, X., Wang, S.L., Zhou, B., Zhang, S.Z., and Liu, D.J. (2005). QTLs for Fusarium head blight

response in a wheat DH population of Wangshuibai/Alondra's'. Euphytica **146,** 183-191.

Jiang, G.L., and Ward, R.W. (2006). Inheritance of resistance to Fusarium head blight in the wheat lines 'CJ 9306' and 'CJ 9403'. Plant Breeding **125,** 417-423.

Jiang, G.L., Shi, J.R., and Ward, R.W. (2007a). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. I. Resistance to fungal spread. Theoretical and Applied Genetics **116,** 3-13.

Jiang, G.L., Dong, Y., Shi, J., and Ward, R.W. (2007b). QTL analysis of resistance to Fusarium head blight in the novel wheat germplasm CJ 9306. II. Resistance to deoxynivalenol accumulation and grain yield loss. Theoretical and Applied Genetics **115,** 1043-1052.

Jiang, G.L., Dong, Y.H., Lewis, J.M., Siler, L., and Ward, R.W. (2006a). Characterization of resistance to *Fusarium graminearum* in a recombinant inbred line population of wheat: resistance to fungal spread, mycotoxin accumulation, and grain yield loss, and trait relationships. Crop Science **46,** 2590-2597.

Jiang, G.L., Chen, Z.X., Xu, Y., Yang, Z.L., Shen, Q.Q., Lu, W.Z., and Ward, R. (2006b). Registration of FHB-resistant and high-yielding wheat germplasms CJ 9403 and CJ 9815. Crop Science **46,** 2724-2726.

Jiang, G.L., Huang, D.C., Shen, Q.L., Yang, Z.L., Lu, W.Z., Shi, J.R., Zhu, H., Chen, Z.X., and Ward, R. (2006c). Registration of wheat germplasms CJ W14 and CJ 9306 highly resistant to fusarium head blight. Crop Science **46,** 2326-2328.

Jiang, G.L., Wu, Z.S., Chen, Z.X., Wu, J.M., Xia, Q.M., Yu, S.R., Wei, X.Z., Shen, Y.J., and Xu, Y. (2007c). Registration of CJ 8809 wheat germplasm with fusarium head blight resistance. Journal of Plant Registrations **1,** 72-74.

Jiao, F., Kawakami, A., and Nakajima, T. (2008). Effects of different carbon sources on trichothecene production and *Tri* gene expression by *Fusarium graminearum* in liquid culture. FEMS Microbiol. Lett. **285,** 212-219.

Jones, D.G., and Clifford, B.C. (1978). Cereal diseases their pathology and control. (Ipswich: BASF UK).

Jones, H.D., Doherty, A., and Wu, H. (2005). Review of methodologies and a protocol for the *Agrobacterium*-mediated transformation of wheat. Plant Methods **1,** 5-13.

Jones, J.D.G., and Dangl, J.L. (2006). The plant immune system. Nature **444,** 323-329.

Jones, R.K. (2000). Assessments of Fusarium head blight of wheat and barley in response to fungicide treatment. Plant Disease **84,** 1021-1030.

Kang, Z., and Buchenauer, H. (1999). Immunocytochemical localization of fusarium toxins in infected wheat spikes by *Fusarium culmorum*. Physiol. Mol. Plant Pathol. **55,** 275-288.

Kang, Z., and Buchenauer, H. (2000a). Ultrastructural and immunocytochemical investigation of pathogen development and host

responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. Physiol. Mol. Plant Pathol. **57,** 255-268.

Kang, Z., and Buchenauer, H. (2000b). Ultrastructural and cytochemical studies on cellulose, xylan and pectin degradation in wheat spikes infected by *Fusarium culmorum*. J. Phytopathol.-Phytopathol. Z. **148,** 263-275.

Kang, Z., and Buchenauer, H. (2002). Immunocytochemical localization of beta-1,3-glucanase and chitinase in *Fusarium culmorum*-infected wheat spikes. Physiol. Mol. Plant Pathol. **60,** 141-153.

Kang, Z., and Buchenauer, H. (2003). Immunocytochemical localization of cell wall-bound thionins and hydroxyproline-rich glycoproteins in *Fusarium culmorum*-infected wheat spikes. J. Phytopathol.-Phytopathol. Z. **151,** 120-129.

Kang, Z.S., and Buchenauer, H. (2000c). Cytology and ultrastructure of the infection of wheat spikes by *Fusarium culmorum*. Mycological Research **104,** 1083-1093.

Kang, Z.S., Buchenauer, H., Huang, L.L., Han, Q.M., and Zhang, H.C. (2008). Cytological and immunocytochemical studies on responses of wheat spikes of the resistant Chinese cv. Sumai 3 and the susceptible cv. Xiaoyan 22 to infection by *Fusarium graminearum*. European Journal of Plant Pathology **120,** 383-396.

Keller, B., and Feuillet, C. (2000). Colinearity and gene density in grass genomes. Trends Plant Sci. **5,** 246-251.

Keller, B., Feuillet, C., and Yahiaoui, N. (2005). Map-based isolation of disease resistance genes from bread wheat: cloning in a supersize genome. Genetical Research **85,** 93-100.

Kelly, J.D., and Vallejo, V. (2005). QTL analysis of mutligenic disease resistance in plant breeding. In Multigenic and induced systemic resistance in plants, S. Tuzun and E. Bent, eds (Springer US.

Keon, J., Antoniw, J., Carzaniga, R., Deller, S., Ward, J.L., Baker, J.M., Beale, M.H., Hammond-Kosack, K., and Rudd, J.J. (2007). Transcriptional adaptation of *Mycosphaerella graminicola* to programmed cell death (PCD) of its susceptible wheat host. Molecular Plant Microbe Interactions **20,** 178-193.

Khan, N.I., Schisler, D.A., Boehm, M.J., Lipps, P.E., and Slininger, P.J. (2004). Field testing of antagonists of Fusarium head blight incited by *Gibberella zeae*. Biol. Control **29,** 245-255.

Khonga, E.B., and Sutton, J.C. (1988). Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Canadian Journal of Plant Pathology **10,** 232-239.

Kim, J.E., Myong, K., Shim, W.B., Yun, S.H., and Lee, Y.W. (2007). Functional characterization of acetylglutamate synthase and phosphoribosylamine-glycine ligase genes in *Gibberella zeae*. Curr. Genet. **51,** 99-108.

Kimura, M., Shingu, Y., Yoneyama, K., and Yamaguchi, I. (1998a). Features of *Tri101*, the trichothecene 3-*O*-acetyltransferase gene, related to the self-defense mechanism in *Fusarium graminearum*. Biosci. Biotechnol. Biochem. **62,** 1033-1036.

Kimura, M., Matsumoto, G., Shingu, Y., Yoneyama, K., and Yamaguchi, I. (1998b). The mystery of the trichothecene 3-*O*-acetyltransferase gene. Analysis of the region around *Tri101* and characterization of its homologue from *Fusarium sporotrichioides* FEBS Lett. **435,** 163-168.

Kimura, M., Kaneko, I., Komiyama, M., Takatsuki, A., Koshino, H., Yoneyama, K., and Yamaguchi, I. (1998c). Trichothecene 3-*O*-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins - Cloning and characterization of *Tri101*. J. Biol. Chem. **273,** 1654-1661.

Kimura, M., Tokai, T., Matsumoto, G., Fujimura, M., Hamamoto, H., Yoneyama, K., Shibata, T., and Yamaguchi, I. (2003a). Trichothecene nonproducer *Gibberella* species have both functional and nonfunctional 3-*O*-acetyltransferase genes. Genetics **163,** 677-684.

Kimura, M., Tokai, T., O'Donnell, K., Ward, T.J., Fujimura, M., Hamamoto, H., Shibata, T., and Yamaguchi, I. (2003b). The trichothecene biosynthesis gene cluster of *Fusarium graminearum* F15 contains a limited number of essential pathway genes and expressed non-essential genes. FEBS Lett. **539,** 105-110.

Klahr, A., Zimmermann, G., Wenzel, G., and Mohler, V. (2007). Effects of environment, disease progress, plant height and heading date on the detection of QTLs for resistance to Fusarium head blight in an European winter wheat cross. Euphytica **154,** 17-28.

Klemsdal, S.S., and Elen, O. (2006). Development of a highly sensitive nested-PCR method using a single closed tube for detection of *Fusarium culmorum* in cereal samples. Lett. Appl. Microbiol. **42,** 544-548.

Klix, M.B., Beyer, M., and Verreet, J.A. (2008). Effects of cultivar, agronomic practices, geographic location, and meteorological conditions on the composition of selected *Fusarium* species on wheat heads. Canadian Journal of Plant Pathology **30,** 46-57.

Kolb, F.L., Bai, G.H., Muehlbauer, G.J., Anderson, J.A., Smith, K.P., and Fedak, G. (2001). Symposium on Genetic Solutions to Fusarium Head Blight in Wheat and Barley: Challenges, Opportunities, and Imperatives. Crop Science **41,** 611-619.

Kruger, W.M., Pritsch, C., Chao, S.M., and Muehlbauer, G.J. (2002). Functional and comparative bioinformatic analysis of expressed genes from wheat spikes infected with *Fusarium graminearum*. Mol. Plant-Microbe Interact. **15,** 445-455.

La Rota, M., and Sorrells, M.E. (2004). Comparative DNA sequence analysis of mapped wheat ESTs reveals the complexity of genome relationships betwen rice and wheat. Functional and Integrative Genomics **4,** 34-46.

Lacey, J., Bateman, G.L., and Mirocha, C.J. (1999). Effects of infection time and moisture on development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat. Ann. Appl. Biol. **134,** 277-283.

Lee, T., Han, Y.K., Kim, K.H., Yun, S.H., and Lee, Y.W. (2002). *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Appl. Environ. Microbiol. **68,** 2148-2154.

Lee, T., Oh, D.W., Kim, H.S., Lee, J., Kim, Y.H., Yun, S.H., and Lee, Y.W. (2001). Identification of deoxynivalenol- and nivalenol-producing

chemotypes of *Gibberella zeae* by using PCR. Appl. Environ. Microbiol. **67,** 2966-2972.

Leisova, L., Kucera, L., Chrpova, J., Sykorova, S., Sip, V., and Ovesna, J. (2006). Quantification of *Fusarium culmorum* in wheat and barley tissues using real-time PCR in comparison with DON content. J. Phytopathol. **154,** 603-611.

Lemmens, M., Buerstmayr, H., Krska, R., Schuhmacher, R., Grausgruber, H., and Ruckenbauer, P. (2004). The effect of inoculation treatment and long-term application of moisture on Fusarium head blight symptoms and deoxynivalenol contamination in wheat grains. European Journal of Plant Pathology **110,** 299-308.

Lemmens, M., Josephs, R., Schuhmacher, R., Grausgruber, H., Buerstmayr, H., Ruckenbauer, P., Neuhold, P., Fidesser, M., and Krska, R. (1997). Head blight (*Fusarium* spp.) on wheat: investigations on the relationship between disease symptoms and mycotoxin content. Cereal Res. Commun. **3/1,** 459-465.

Lemmens, M., Scholz, U., Berthiller, F., Dall'Asta, C., Koutnik, A., Schuhmacher, R., Adam, G., Buerstmayr, H., Mesterhazy, A., Krska, R., and Ruckenbauer, P. (2005). The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major quantitative trait locus for Fusarium head blight resistance in wheat. Mol. Plant-Microbe Interact. **18,** 1318-1324.

Li, C., Zhu, H., Zhang, C., Lin, F., Xue, S., Cao, Y., Zhang, Z., Zhang, L., and Ma, Z. (2008). Mapping QTLs associated with Fusarium-damaged kernels in the Nanda 2419 x Wangshuibai population. Euphytica **163,** 185-191.

Li, W.L., Faris, J.D., Muthukrishnan, S., Liu, D.J., Chen, P.D., and Gill, B.S. (2001). Isolation and characterization of novel cDNA clones of acidic chitinases and beta-1,3-glucanases from wheat spikes infected by *Fusarium graminearum*. Theoretical and Applied Genetics **102,** 353-362.

Lin, F., Kong, Z.X., Zhu, H.L., Xue, S.L., Wu, J.Z., Tian, D.G., Wei, J.B., Zhang, C.Q., and Ma, Z.Q. (2004). Mapping QTL associated with resistance to *Fusarium* head blight in the Nanda2419 x Wangshuibai population. I. Type II resistance. Theoretical and Applied Genetics **109,** 1504-1511.

Lin, F., Xue, S.L., Zhang, Z.Z., Zhang, C.Q., Kong, Z.X., Yao, G.Q., Tian, D.G., Zhu, H.L., Li, C.J., Cao, Y., Wei, J.B., Luo, Q.Y., and Ma, Z.Q. (2006). Mapping QTL associated with resistance to Fusarium head blight in the Nanda2419 x Wangshuibai population. II: Type I resistance. Theoretical and Applied Genetics **112,** 528-535.

Lis, J.T., Simon, J.A., and Sutton, C.A. (1983). New heat shock puffs and beta-galactosidase activity resulting from transformation of Drosophila with an *hsp70-lacz* hybrid gene. Cell **35,** 403-410.

Liu, S., Yu, K., and Park, S.J. (2008). Development of STS markers and QTL validation for common bacterial blight resistance in common bean. Plant Breeding **127,** 62-68.

Liu, S., Zhang, X., Pumphrey, M.O., Stack, R.W., Gill, B.S., and Anderson, J.A. (2006). Complex microlinearity among wheat, rice, and barley

revealed by fine mapping of the genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Functional and Integrative Genomics **6,** 83-89.

Liu, S., Abate, Z.A., Lu, H., Musket, T., Davis, G.L., and McKendry, A.L. (2007). QTL associated with Fusarium head blight resistance in the soft red winter wheat Ernie. Theoretical and Applied Genetics **115,** 417-427.

Liu, S.X., and Anderson, J.A. (2003a). Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Science **43,** 760-766.

Liu, S.X., and Anderson, J.A. (2003b). Targeted molecular mapping of a major wheat QTL for *Fusarium* head blight resistance using wheat ESTs and synteny with rice. Genome **46,** 817-823.

Liu, W.Z., Langseth, W., Skinnes, H., Elen, O.N., and Sundheim, L. (1997). Comparison of visual head blight ratings, seed infection levels, and deoxynivalenol production for assessment of resistance in cereals inoculated with *Fusarium culmorum*. European Journal of Plant Pathology **103,** 589-595.

Lovell, D.J., Powers, S.J., Welham, S.J., and Parker, S.R. (2004). A perspective on the measurement of time in plant disease epidemiology. Plant Pathology **53,** 705-712.

Lu, S.W., Kroken, S., Lee, B.N., Robbertse, B., Churchill, A.C., Yoder, O.C., and Turgeon, B.G. (2003). A novel class of gene controlling virulence in plant pathogenic ascomycete fungi. Proc. Natl. Acad. Sci. U. S. A. **100,** 5980-5985.

Lucas, J.A. (1998). Plant Pathology and Plant Pathogens. (Oxford: Blackwell Publishing).

Lucas, J.A., Dyer, P.S., and Murray, T.D. (2000). Pathogenicity, host-specificity, and population biology of *Tapesia* spp., causal agents of eyespot disease of cereals. Advances in Botanical Research **33,** 225-258.

Luongo, L., Galli, M., Corazza, L., Meekes, E., De Haas, L., Van der Plas, C.L., and Kohl, J. (2005). Potential of fungal antagonists for biocontrol of *Fusarium* spp. in wheat and maize through competition in crop debris. Biocontrol Sci. Technol. **15,** 229-242.

Ma, H.X., Bai, G.H., Zhang, X., and Lu, W.Z. (2006a). Main effects, epistasis, and environmental interactions of quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population. Phytopathology **96,** 534-541.

Ma, H.X., Zhang, K.M., Gao, L., Bai, G.H., Chen, H.G., Cai, Z.X., and Lu, W.Z. (2006b). Quantitative trait loci for resistance to Fusarium head blight and deoxynivalenol accumulation in Wangshuibai wheat under field conditions. Plant Pathology **55,** 739-745.

Mackintosh, C.A., Garvin, D.F., Radmer, L.E., Heinen, S.J., and Muehlbauer, G.J. (2006). A model wheat cultivar for transformation to improve resistance to Fusarium head blight. Plant Cell Reports **25,** 313-319.

Maier, F.J., Miedaner, T., Hadeler, B., Felk, A., Salomon, S., Lemmens, M., Kassner, H., and Schafer, W. (2006). Involvement of trichothecenes in fusarioses of wheat, barley and maize evaluated by gene disruption of

the trichodiene synthase (Tri5) gene in three field isolates of different chemotype and virulence. Mol. Plant Pathol. **7,** 449-461.

Makandar, R., Essig, J.S., Schapaugh, M.A., Trick, H.N., and Shah, J. (2006). Genetically engineered resistance to Fusarium head blight in wheat by expression of *Arabidopsis NPR1*. Mol. Plant-Microbe Interact. **19,** 123-129.

Mardi, M., Buerstmayr, H., Ghareyazie, B., Lemmens, M., Mohammadi, S.A., Nolz, R., and Ruckenbauer, P. (2005). QTL analysis of resistance to Fusarium head blight in wheat using a 'Wangshuibai'-derived population. Plant Breeding **124,** 329-333.

Maresca, M., Mahfoud, R., Garmy, N., and Fantini, J. (2002). The mycotoxin deoxynivalenol affects nutrient absorption in human intestinal epithelial cells. J. Nutr. **132,** 2723-2731.

Markell, S.G., and Francl, L.J. (2003). Fusarium head blight inoculum: species prevalence and *Gibberella zeae* spore type. Plant Disease **87,** 814-820.

Martin, R.A., and Johnston, H.W. (1982). Effects and control of fusarium diseases of cereal grains in the Atlantic Provinces. Canadian Journal of Plant Pathology **4,** 210-216.

McCallum, C.M., Comai, L., Greene, E.A., and Henikoff, S. (2000). Targeting Induced Local Lesions In Genomes (TILLING) for plant functional genomics. Plant Physiol. **123,** 439-442.

McCartney, C.A., Somers, D.J., Fedak, G., and Cao, W. (2004). Haplotype diversity at fusarium head blight resistance QTLs in wheat. Theoretical and Applied Genetics **109,** 261-271.

McCartney, C.A., Somers, D.J., Fedak, G., DePauw, R.M., Thomas, J., Fox, S.L., Humphreys, D.G., Lukow, O., Savard, M.E., McCallum, B.D., Gilbert, J., and Cao, W. (2007). The evaluation of FHB resistance QTLs introgressed into elite Canadian spring wheat germplasm. Mol. Breed. **20,** 209-221.

McCormick, S.P., and Hohn, T.M. (1997). Accumulation of trichothecenes in liquid cultures of a *Fusarium sporotrichioides* mutant lacking a functional trichothecene C-15 hydroxylase. Appl. Environ. Microbiol. **63,** 1685-1688.

McCormick, S.P., and Alexander, N.J. (2002). *Fusarium Tri8* encodes a trichothecene C-3 esterase. Appl. Environ. Microbiol. **68,** 2959-2964.

McCormick, S.P., Hohn, T.M., and Desjardins, A.E. (1996). Isolation and characterization of *Tri3*, a gene encoding 15-*O*-acetyltransferase from *Fusarium sporotrichioides*. Appl. Environ. Microbiol. **62,** 353-359.

McCormick, S.P., Alexander, N.J., and Proctor, R.H. (2006a). Heterologous expression of two trichothecene P450 genes in *Fusarium verticillioides*. Can. J. Microbiol. **52,** 220-226.

McCormick, S.P., Alexander, N.J., and Proctor, R.H. (2006b). Fusarium *Tri4* encodes a multifunctional oxygenase required for trichothecene biosynthesis. Can. J. Microbiol. **52,** 636-642.

McCormick, S.P., Alexander, N.J., Trapp, S.E., and Hohn, T.M. (1999). Disruption of *TRI101*, the gene encoding trichothecene 3-*O*-acetyltransferase, from *Fusarium sporotrichioides*. Appl. Environ. Microbiol. **65,** 5252-5256.

McCormick, S.P., Harris, L.J., Alexander, N.J., Ouellet, T., Saparno, A., Allard, S., and Desjardins, A.E. (2004). *Tri1* in *Fusarium graminearum* encodes a P450 oxygenase. Appl. Environ. Microbiol. **70,** 2044-2051.

McDonald, B.A., and Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. **40,** 349-379.

McDowell, J.M., and Simon, S.A. (2006). Recent insights into *R* gene evolution. Mol. Plant Pathol. **7,** 437-448.

McKeehen, J.D., Busch, R.H., and Fulcher, R.G. (1999). Evaluation of wheat (*Triticum aestivum* L.) phenolic acids during grain development and their contribution to *Fusarium* resistance. J. Agric. Food Chem. **47,** 1476-1482.

McKendry, A.L., Berg, J.E., Tague, D.N., and Kephart, K.D. (1995). Registration of 'Ernie' wheat. Crop Science **35,** 1513.

McKendry, A.L., Tague, D.N., Wright, R.L., and Tremain, J.A. (2007). Registration of 'Bess' wheat. Journal of Plant Registrations **1,** 21-23.

McMullen, M., Jones, R., and Gallenberg, D. (1997). Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease **81,** 1340-1348.

Meek, I.B., Peplow, A.W., Ake, C., Phillips, T.D., and Beremand, M.N. (2003). *Tri1* encodes the cytochrome P450 monooxygenase for C-8 hydroxylation during trichothecene biosynthesis in *Fusarium sporotrichioides* and resides upstream of another new *Tri* gene. Appl. Environ. Microbiol. **69,** 1607-1613.

Mergoum, M., Frohberg, R.C., and Stack, R.W. (2007). Registration of spring wheat germplasm ND 751 resistant to Fusarium head blight. Crop Science **47,** 455-457.

Mergoum, M., Frohberg, R.C., Miller, J.D., Rasmussen, J.B., and Stack, R.W. (2005). Registration of spring wheat germplasm ND 744 resistant to fusarium head blight, leaf and stem rusts. Crop Science **45,** 430-431.

Mergoum, M., Frohberg, R.C., Stack, R.W., Olson, T., Friesen, T.L., and Rasmussen, J.B. (2006). Registration of 'Glenn' wheat. Crop Science **46,** 473-474.

Mergoum, M., Frohberg, R.C., Stack, R.W., Singh, P.K., Ali, S., Rasmussen, J.W., Friesen, T.L., and Adhikari, T.B. (2008). Registration of spring wheat germplasm ND 756 combining resistances to foliar diseases and Fusarium head blight. Journal of Plant Registrations **2,** 61-64.

Mesterhazy, A. (1995). Types and components of resistance to Fusarium head blight of wheat. Plant Breeding **114,** 377-386.

Mesterhazy, A. (2002). Role of deoxynivalenol in aggressiveness of *Fusarium graminearum* and *F. culmorum* and in resistance to Fusarium head blight. European Journal of Plant Pathology **108,** 675-684.

Mesterhazy, A., Bartok, T., and Lamper, C. (2003). Influence of wheat cultivar, species of *Fusarium*, and isolate aggressiveness on the efficacy of fungicides for control of Fusarium head blight. Plant Disease **87,** 1107-1115.

Mesterhazy, A., Bartok, T., Mirocha, C.G., and Komoroczy, R. (1999). Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. Plant Breeding **118,** 97-110.

Mesterhazy, A., Bartok, T., Kaszonyi, G., Varga, M., Toth, B., and Varga, J. (2005). Common resistance to different *Fusarium* spp. causing *Fusarium* head blight in wheat. European Journal of Plant Pathology **112,** 267-281.

Miedaner, T., and Perkowski, J. (1996). Correlations among *Fusarium culmorum* head blight resistance, fungal colonization and mycotoxin contents in winter rye. Plant Breeding **115,** 347-351.

Miedaner, T., and Voss, H.-H. (2008). Effect of dwarfing *Rht* genes on Fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Science **48,** 2115-2122.

Miedaner, T., Moldovan, A., and Ittu, M. (2003a). Comparison of spray and point inoculation to assess resistance to Fusarium head blight in a multienvironment wheat trial. Phytopathology **93,** 1068-1072.

Miedaner, T., Schneider, B., and Geiger, H.H. (2003b). Deoxynivalenol (DON) content and Fusarium head blight resistance in segregating populations of winter rye and winter wheat. Crop Science **43,** 519-526.

Miedaner, T., Reinbrecht, C., Lauber, U., Schollenberger, M., and Geiger, H.H. (2001). Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to Fusarium head blight in rye, triticale, and wheat. Plant Breeding **120,** 97-105.

Miedaner, T., Heinrich, N., Schneider, B., Oettler, G., Rohde, S., and Rabenstein, F. (2004). Estimation of deoxynivalenol (DON) content by symptom rating and exoantigen content for resistance selection in wheat and triticale. Euphytica **139,** 123-132.

Miedaner, T., Wilde, F., Steiner, B., Buerstmayr, H., Korzun, V., and Ebmeyer, E. (2006). Stacking quantitative trait loci (QTL) for Fusarium head blight resistance from non-adapted sources in an European elite spring wheat background and assessing their effects on deoxynivalenol (DON) content and disease severity. Theoretical and Applied Genetics **112,** 562-569.

Miller, J.D., and Young, J.C. (1985). Deoxynivalenol in an experimental *Fusarium graminearum* infection of wheat. Canadian Journal of Plant Pathology **7,** 132-134.

Miller, J.D., and Greenhalgh, R. (1985). Nutrient effects on the biosynthesis of trichothecenes and other metabolites by *Fusarium graminearum*. Mycologia **77,** 130-136.

Miller, J.D., and Arnison, P.G. (1986). Degradation of deoxynivalenol by suspension cultures of the Fusarium head blight resistant wheat cultivar Frontana. Canadian Journal of Plant Pathology **8,** 147-150.

Miller, J.D., and Ewen, M.A. (1997). Toxic effects of deoxynivaelonol on ribosomes and tissues of the spring wheat cultivars Frontana and Casavant. Natural Toxins **5,** 234-237.

Miller, J.D., Young, J.C., and Sampson, D.R. (1985). Deoxynivalenol and Fusarium head blight resistance in spring cereals. Phytopathologische Zeitschrift-Journal of Phytopathology **113,** 359-367.

Miller, J.D., Greenhalgh, R., Wang, Y.Z., and Lu, M. (1991). Trichothecene chemotypes of three *Fusarium* species. Mycologia **83,** 121-130.

Milus, E.A., Kristensen, K., and Hovmoller, M.S. (2009). Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp. *tritici* causing stipe rust of wheat. Phytopathology **99,** 89-94.

Mirocha, C.J., Yu, H., Evans, C.K., Kolaczowski, E.K., and Dill-Macky, R. (1997). Chemistry and physiology of deoxynivalenol in pathogenesis. Cereal Res. Commun. **25,** 309-313.

Monke, E., and Schafer, W. (1993). Transient and stable gene expression inthe fungal maize pathogen *Cochliobolus heterostrophus* after transformation with the beta-glucuronidase (GUS) gene. Mol. Gen. Genet. **241,** 73-80.

Moore, G. (1995). Cereal genome evolution: pastoral pursuits with 'Lego' genomes. Curr. Biol. **5,** 717-724.

Moore, G., Gale, M.D., Kurata, N., and Flavell, R.B. (1993). Molecular analysis of small grain cereal genomes: current status and prospects. Nat. Biotechnol. **11,** 584-589.

Moore, G., Devos, K.M., Wang, Z.H., and Gale, M.D. (1995). Grasses, line up and form a circle. Curr. Biol. **5,** 737-739.

Moss, M.O. (2002). Mycotoxin review - 2. *Fusarium*. Mycologist **16,** 158-161.

Najaphy, A., Toorchi, M., Mohammadi, S.A., Chalmers, K.J., Moghaddam, M., Torabi, M., and Aharizad, S. (2006). Identification of Fusarium head blight resistance QTLs in a wheat population using SSR markers. Biotechnology **5,** 222-227.

Nass, H.G., Caldwell, C.D., and Price, M.A. (2006). Nass hard red spring wheat. Can. J. Plant Sci. **86,** 493-495.

Newell, S.Y., Miller, J.D., and Fallon, R.D. (1987). Ergosterol content of salt-marsh fungi: effect of growth conditions and mycelial age. Mycologia **79,** 688-695.

Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W., and Joyce, D. (1998). Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol. Mol. Plant Pathol. **53,** 17-37.

Nicholson, P., Doohan, F., Joyce, D., Rezanoor, H.N., Simpson, D., Smith, P.H., Turner, A., and Weston, G. (1997). Detection and quantification of individual fungal species in Fusarium disease complexes of cereals by PCR. Cereal Res. Commun. **25,** 40-46.

Niessen, M.L., and Vogel, R.F. (1997). Specific identification of *Fusarium graminearum* by PCR with *gao*A targeted primers. Systematic and Applied Microbiology **20,** 111-113.

Nightingale, M.J., Marchylo, B.A., Clear, R.M., Dexter, J.E., and Preston, K.R. (1999). Fusarium head blight: effect of fungal proteases on wheat storage proteins. Cereal Chem. **76,** 150-158.

Nurnberger, T., Brunner, F., Kemmerling, B., and Piater, L. (2004). Innate immunity in plants and animals: striking similarites and obvious differences. Immunological Reviews **198,** 249-266.

Nyvall, R.F. (1970). Chlamydospores of Fusarium roseum 'Graminearum' as survival structures. Phytopathology **60,** 1175-1177.

O'Donnell, K., Kistler, H.C., Tacke, B.K., and Casper, H.H. (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. U. S. A. **97,** 7905-7910.

O'Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C., and Aoki, T. (2004). Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet. Biol. **41,** 600-623.

O'Neill, K., Damoglou, A.P., and Patterson, M.F. (1993). Toxin production by *Fusarium culmorum* IMI 309344 and *F. graminearum* NRRL 5883 on grain substrates. Journal of Applied Bacteriology **74,** 625-628.

Ochiai, N., Tokai, T., Takahashi-Ando, N., Fujimura, M., and Kimura, M. (2007). Genetically engineered Fusarium as a tool to evaluate the effects of environmental factors on initiation of trichothecene biosynthesis. FEMS Microbiol. Lett. **275,** 53-61.

Oelke, L.M., and Kolmer, J.A. (2005). Genetics of leaf rust resistance in spring wheat cultivars alsen and norm. Phytopathology **95,** 773-778.

Oerke, E.C. (2006). Crop losses to pests. J. Agric. Sci. **144,** 31-43.

Oide, S., Moeder, W., Krasnoff, S., Gibson, D., Haas, H., Yoshioka, K., and Turgeon, B.G. (2006). *NPS6*, encoding a nonribosomal peptide synthetase involved in siderophore-mediated iron metabolism, is a conserved virulence determinant of plant pathogenic ascomycetes. The Plant Cell **18,** 2836-2853.

Oliver, R.P., Farman, M.L., Jones, J.D.G., and Hammond-Kosack, K.E. (1993). Use of fungal transformants expressing Beta-glucuronidase activity to detect infection and measure hyphal biomass in infected plant tissues. Mol. Plant-Microbe Interact. **6,** 521-525.

Opanowicz, M., Vain, P., Draper, J., Parker, D., and Doonan, J.H. (2008). *Brachypodium distachyon*: making hay with a wild grass. Trends Plant Sci. **13,** 172-177.

Osborne, L.E., and Stein, J.M. (2007). Epidemiology of Fusarium head blight on small-grain cereals. International Journal of Food Microbiology **119,** 103-108.

Ow, D.W., Wood, K.V., DeLuca, M., de Wet, J.R., Helinski, D.R., and Howell, S.H. (1986). Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science **234,** 856-859.

Paillard, S., Schnurbusch, T., Tiwari, R., Messmer, M., Winzeler, M., Keller, B., and Schachermayr, G. (2004). QTL analysis of resistance to Fusarium head blight in Swiss winter wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics **109,** 323-332.

Palmer, C.L., and Skinner, W. (2002). *Mycosphaerella graminicola*: latent infection, crop devastation and genomics. Mol. Plant Pathol. **3,** 63-70.

Park, Y.S., Choi, I.D., Kang, C.M., Ham, M.S., Kim, J.H., Kim, T.H., Yun, S.H., Lee, Y.W., Chang, H.I., Sung, H.C., and Yun, C.W. (2006).

Functional identification of high-affinity iron permeases from *Fusarium graminearum*. Fungal Genet. Biol. **43,** 273-282.

Parry, D.W., Bayles, R.A., and Preistley, R.H. (1984). Resistance of winter wheat varieties to ear blight (*Fusarium culmorum*). Journal of the National Institute of Agricultural Botany **16,** 465-468.

Parry, D.W., Jenkinson, P., and McLeod, L. (1995). Fusarium ear blight (Scab) in small grain cereals - a review. Plant Pathology **44,** 207-238.

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E., and Tanksley, S.D. (1988). Resolution of quantitative traits into mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature **335,** 721-726.

Paul, P.A., Lipps, P.E., Hershman, D.E., McMullen, M.P., Draper, M.A., and Madden, L.V. (2008). Efficacy of triazole-based fungicides for fusarium head blight and deoxynivalenol control in wheat: a multivariate meta-analysis. Phytopathology **98,** 999-1011.

Paux, E., Sourdille, P., Salse, J., Saintenac, C., Choulet, F., Leroy, P., Korol, A., Michalak, M., Kianian, S., Spielmeyer, W., Lagudah, E., Somers, D., Killian, A., Alaux, M., Vautrin, S., Berges, H., Eversole, K., Appels, R., Safar, J., Simkova, H., Dolezel, J., Bernard, M., and Feuillet, C. (2008). A physical map of the 1-gigabase bread wheat chromosome 3B. Science **322,** 101-104.

Payne, R.W., Harding, S.A., Murray, D.A., Soutar, D.M., Baird, D.B., Welham, S.J., Kane, A.F., Gilmour, A.R., Thompson, R., Webster, R., and Tunnicliffe Wilson, G. (2005). The Guide to GenStat Release 8, Part 2: Statistics. (Oxford: VSN International).

Peart, J.R., Cook, G., Feys, B.J., Parker, J.E., and Baulcombe, D.C. (2002a). An *EDS1* orthologue is required for *N*-mediated resistance against tobacco mosaic virus. The Plant Journal **29,** 569-579.

Peart, J.R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D.C., Schauser, L., Jaggard, D.A.W., Xiao, S., Coleman, M.J., Dow, M., Jones, J.D.G., Shirasu, K., and Baulcombe, D.C. (2002b). Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. Proc. Natl. Acad. Sci. U. S. A. **99,** 10865-10869.

Peplow, A.W., Tag, A.G., Garifullina, G.F., and Beremand, M.N. (2003a). Identification of new genes positively regulated by *Tri10* and a regulatory network for trichothecene mycotoxin production. Appl. Environ. Microbiol. **69,** 2731-2736.

Peplow, A.W., Meek, I.B., Wiles, M.C., Phillips, T.D., and Beremand, M.N. (2003b). *Tri16* is required for esterification of position C-8 during trichothecene mycotoxin production by *Fusarium sporotrichioides*. Appl. Environ. Microbiol. **69,** 5935-5940.

Pereyra, S.A., and Dill-Macky, R. (2008). Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to Fusarium head blight inoculum. Plant Disease **92,** 800-807.

Pinson-Gadais, L., Richard-Forget, F., Frasse, P., Barreau, C., Cahagnier, B., Richard-Molard, D., and Bakan, B. (2008). Magnesium represses trichothecene biosynthesis and modulates *Tri5*, *Tri6*, and *Tri12* genes expression in *Fusarium graminearum*. Mycopathologia **165,** 51-59.

Pirgozliev, S.R., Ray, R.V., Edwards, S.G., Hare, M.C., and Jenkinson, P. (2008). Effect of timing of fungicide application on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in winter wheat grain. Cereal Res. Commun. **36,** 289-299.

Plassard, C.S., Mousain, D.G., and Salsac, L.E. (1982). Estimation of mycelial growth of basidiomycetes by means of chitin determination. Phytochemistry **21,** 345-348.

Ponts, N., Pinson-Gadais, L., and Richard-Forget, F. (2003). H_2O_2 effects on Trichothecenes B (DON, ADON) production by *Fusarium graminearum* in liquid culture. Aspects of Applied Biology **68,** 223-228.

Ponts, N., Pinson-Gadais, L., Verdal-Bonnin, M.N., Barreau, C., and Richard-Forget, F. (2006). Accumulation of deoxynivalenol and its 15-acetylated form is significantly modulated by oxidative stress in liquid cultures of *Fusarium graminearum*. FEMS Microbiol. Lett. **258,** 102-107.

Ponts, N., Pinson-Gadais, L., Barreau, C., Richard-Forget, F., and Ouellet, T. (2007). Exogenous H_2O_2 and catalase treatments interfere with *Tri* genes expression in liquid cultures of *Fusarium graminearum*. FEBS Lett. **581,** 443-447.

Pozar, G., Butruille, D., Silva, H.D., Patterson McCuddin, Z., and Penna, J.C.V. (2009). Mapping and validation of quantitative trait loci for resistance to *Cercospora zeae-maydis* infection in tropical maize. Theoretical and Applied Genetics **118,** 553-564.

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A., and Vance, C.P. (2000). Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. Mol. Plant-Microbe Interact. **13,** 159-169.

Pritsch, C., Vance, C.P., Bushnell, W.R., Somers, D.A., Hohn, T.M., and Muehlbauer, G.J. (2001). Systemic expression of defense response genes in wheat spikes as a response to *Fusarium graminearum* infection. Physiol. Mol. Plant Pathol. **58,** 1-12.

Proctor, R.H., Hohn, T.M., and McCormick, S.P. (1995a). Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Mol. Plant-Microbe Interact. **8,** 593-601.

Proctor, R.H., Hohn, T.M., McCormick, S.P., and Desjardins, A.E. (1995b). *Tri6* encodes an unusual zinc finger protein involved in regulation of trichothecene biosynthesis in *Fusarium Sporotrichioides*. Appl. Environ. Microbiol. **61,** 1923-1930.

Proctor, R.H., Desjardins, A.E., McCormick, S.P., Plattner, R.D., Alexander, N.J., and Brown, D.W. (2002). Genetic analysis of the role of trichothecene and fumonisin mycotoxins in the virulence of *Fusarium*. European Journal of Plant Pathology **108,** 691-698.

Pumphrey, M.O., Bernardo, R., and Anderson, J.A. (2007). Validating the Fhb1 QTL for fusarium head blight resistance in near-isogenic wheat lines developed from breeding populations. Crop Science **47,** 200-206.

Ramamoorthy, V., Zhao, X., Snyder, A.K., Xu, J.R., and Shah, D.M. (2007a). Two mitogen-activated protein kinase signalling cascades mediate basal resistance to antifungal plant defensins in *Fusarium graminearum*. Cellular Microbiology **9,** 1491-1506.

Ramamoorthy, V., Cahoon, E.B., Li, J., Thokala, M., Minto, R.E., and Shah, D.M. (2007b). Glucosylceramide synthase is essential for alfalfa defensin-meidated growth inhibition but not for pathogenicity of *Fusarium graminearum*. Mol. Microbiol. **66,** 771-786.

Rep, M., Dekker, H.L., Vossen, J.H., de Boer, A.D., Houterman, P.M., Speijer, D., Back, J.W., de Koster, C.G., and Cornelissen, B.J.C. (2002). Mass spectrometric identification of isoforms of PR proteins in xylem sap of fungus-infected tomato. Plant Physiol. **130,** 904-917.

Ribichich, K.F., Lopez, S.E., and Vegetti, A.C. (2000). Histopathological spikelet changes produced by *Fusarium graminearum* in susceptible and resistant wheat cultivars. Plant Disease **84,** 794-802.

Richard, G., Bailey, J.A., Keon, J.P.R., and Hargreaves, J.A. (1992). Development of a GUS reporter gene system for the maize pathogen *Ustilago maydis*. Physiol. Mol. Plant Pathol. **40,** 383-393.

Roberts, I.N., Oliver, R.P., Punt, P.J., and van den Hondel, C.A.M.J.J. (1989). Expression of the *Escherichia coli* beta-glucuronidase gene in industrial and phytopathogenic filamentous fungi. Curr. Genet. **15,** 177-180.

Rocha, O., Ansari, K., and Doohan, F.M. (2005). Effects of trichothecene mycotoxins on eukaryotic cells: a review. Food Addit. Contam. **22,** 369-378.

Roder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P., and Ganal, M.W. (1998). A microsatellite map of wheat. Genetics **149,** 2007-2023.

Rotter, B.A., Prelusky, D.B., and Pestka, J.J. (1996). Toxicology of deoxynivalenol (vomitoxin). J. Toxicol. Environ. Health **48,** 1-34.

Ruckenbauer, P., Buerstmayr, H., and Lemmens, M. (2001). Present strategies in resistance breeding against scab (*Fusarium* spp.). Euphytica **119,** 121-127.

Rudd, J.C., Horsley, R.D., McKendry, A.L., and Elias, E.M. (2001). Host plant resistance genes for Fusarium head blight: sources, mechanisms, and utility in conventional breeding systems. Crop Science **41,** 620-627.

Saghai Maroof, M.A., Soliman, K.M., Jorgensen, R.A., and Allard, R.W. (1984). Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. U. S. A. **81,** 8014-8018.

Savard, M.E., Sinha, R.C., Seaman, W.L., and Fedak, G. (2000). Sequential distribution of the mycotoxin deoxynivalenol in wheat spikes after inoculation with *Fusarium graminearum*. Canadian Journal of Plant Pathology **22,** 280-285.

Savitch, L.V., Subramaniam, R., Allard, G.C., and Singh, J. (2007). The GLK1 'regulon' encodes disease defense related proteins and confers resistance to *Fusarium graminearum* in Arabidopsis. Biochem. Biophys. Res. Commun. **359,** 234-238.

Schaafsma, A.W., Tamburic-Ilinic, L., Miller, J.D., and Hooker, D.C. (2001). Agronomic considerations for reducing deoxynivalenol in wheat grain. Canadian Journal of Plant Pathology **23,** 279-285.

Schilling, A.G., Moller, E.M., and Geiger, H.H. (1996). Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum*, *F. graminearum*, and *F. avenaceum*. Phytopathology **86,** 515-522.

Schmolke, M., Zimmermann, G., Schweizer, G., Miedaner, T., Korzun, V., Ebmeyer, E., and Hartl, L. (2008). Molecular mapping of quantitative trait loci for field resistance to Fusarium head blight in a European winter wheat population. Plant Breeding **127,** 459-464.

Schmolke, M., Zimmermann, G., Buerstmayr, H., Schweizer, G., Miedaner, T., Korzun, V., Ebmeyer, E., and Hartl, L. (2005). Molecular mapping of Fusarium head blight resistance in the winter wheat population Dream/Lynx. Theoretical and Applied Genetics **111,** 747-756.

Schroeder, H.W., and Christensen, J.J. (1963). Factors affecting resistance of wheat to scab caused by Gibberella zeae. Phytopathology **53,** 831-838.

Schulman, A.H., Gupta, P.K., and Varshney, R.K. (2004). Organization of retrotransposons and microsatellites in cereal genomes. In Cereal Genomics, P.K. Gupta and R.K. Varshney, eds (London: Kluwer Academic Publishers), pp. 83-118.

Schwarz, P.B., Beattie, S., and Casper, H.H. (1996). Relationship between *Fusarium* infestation of barley and the gushing potential of malt. J. Inst. Brew. **102,** 93-96.

Scofield, S.R., Huang, L., Brandt, A.S., and Gill, B.S. (2005). Development of a virus-induced gene-silencing system for hexaploid wheat and its use in functional analysis of the *Lr21*-mediated leaf rust resistance pathway. Plant Physiol. **138,** 2165-2173.

Scott, P.M., Nelson, K., Kanhere, S.R., Karpinski, K.F., Hayward, S., Neish, G.A., and Teich, A.H. (1984). Decline in deoxynivalenol (vomitoxin) concentrations in 1983 Ontario winter wheat before harvest. Appl. Environ. Microbiol. **48,** 884-886.

Sears, R.G., Moffatt, J.M., Martin, T.J., Cox, T.S., Bequette, R.K., Curran, S.P., Chung, O.K., Heer, W.F., Long, J.H., and Witt, M.D. (1997). Registration of 'Jagger' wheat. Crop Science **37,** 1010.

Semagn, K., Skinnes, H., Bjornstad, A., Maroy, A.G., and Tarkegne, Y. (2007). Quantitative trait loci controlling Fusarium head blight resistance and low deoxynivalenol content in hexaploid wheat population from 'Arina' and NK93604. Crop Science **47,** 294-303.

Seong, K., Hou, Z.M., Tracy, M., Kistler, H.C., and Xu, J.R. (2005). Random insertional mutagenesis identifies genes associated with virulence in the wheat scab fungus *Fusarium graminearum*. Phytopathology **95,** 744-750.

Seong, K., Li, L., Hou, Z.M., Tracy, M., Kistler, H.C., and Xu, J.R. (2006). Cryptic promoter activity in the coding region of the HMG-CoA reductase gene in *Fusarium graminearum*. Fungal Genet. Biol. **43,** 34-41.

Seong, K.Y., Zhao, X., Xu, J.R., Guldener, U., and Kistler, H.C. (2008). Conidial germination in the filamentous fungus *Fusarium graminearum*. Fungal Genet. Biol. **45,** 389-399.

Seong, K.Y., Pasquali, M., Zhou, X., Song, J., Hilburn, K., McCormick, S., Dong, Y., Xu, J.R., and Kistler, H.C. (2009). Global gene regulation by

Fusarium transcription factors *Tri6* and *Tri10* reveals adaptations for toxin biosynthesis. Mol. Microbiol. **72,** 354-367.

Shaner, G., and Finney, R.E. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. Phytopathology **67,** 1051-1056.

Shen, X., Zhou, M., Lu, W., and Ohm, H. (2003a). Detection of Fusarium head blight resistance QTL in a wheat population using bulked segregant analysis. Theoretical and Applied Genetics **106,** 1041-1047.

Shen, X.R., Ittu, M., and Ohm, H.W. (2003b). Quantitative trait loci conditioning resistance to Fusarium head blight in wheat line F201R. Crop Science **43,** 850-857.

Shi, J.R., Xu, D.H., Yang, H.Y., Lu, Q.X., and Ban, T. (2008). DNA marker analysis for pyramided of Fusarium head blight (FHB) resistance QTLs from different germplasm. Genetica **133,** 77-84.

Shim, W.B., Sagaram, U.S., Choi, Y.E., So, J., Wilkinson, H.H., and Lee, Y.W. (2006). *FSR1* is essential for virulence and female fertility in *Fusarium verticillioides* and *F. graminearum*. Mol. Plant-Microbe Interact. **19,** 725-733.

Silva, L.O., Singh, S.P., and Pastor-Corrales, M.A. (1989). Inheritance to bacterial blight in common bean. Theoretical and Applied Genetics **78,** 619-624.

Simpson, D.R., Weston, G.E., Turner, J.A., Jennings, P., and Nicholson, P. (2001). Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. European Journal of Plant Pathology **107,** 421-431.

Singh, R.P., Ma, H., and Rajaram, S. (1995). Genetic analysis of resistance to scab in spring wheat cultivar frontana. Plant Disease **79,** 238-240.

Sinha, R.C., and Savard, M.E. (1997). Concentration of deoxynivalenol in single kernels and various tissues of wheat heads. Canadian Journal of Plant Pathology **19,** 8-12.

Sip, V., Chrpova, J., Leisova, L., Sykorova, S., Kucera, L., and Ovesna, J. (2007). Effects of genotype, environment and fungicide treatment on development of Fusarium head blight and accumulation of DON in winter wheat grain Czech Journal of Genetics and Plant Breeding **43,** 16-31.

Sip, V., Bartos, P., Chrpova, J., Hanzalova, A., Sirlova, L., Sarova, J., Dumalasova, V., Cejka, L., Hanisova, A., Bobkova, L., Bizova, I., and Horcicka, P. (2005). Theoretical bases and sources for breeding wheat for combined disease resistance. Czech Journal of Genetics and Plant Breeding **41,** 127-143.

Siranidou, E., Kang, Z., and Buchenauer, H. (2002). Studies on symptom development, phenolic compounds and morphological defence responses in wheat cultivars differing in resistance to *Fusarium* head blight. J. Phytopathol.-Phytopathol. Z. **150,** 200-208.

Skadsen, R.W., and Hohn, T.A. (2004). Use of *Fusarium graminearum* transformed with *gfp* to follow infection patterns in barley and *Arabidopsis*. Physiol. Mol. Plant Pathol. **64,** 45-53.

Skov, J., Lemmens, M., and Giese, H. (2004). Role of a *Fusarium culmorum* ABC transporter (*FcABC1*) during infection of wheat and barley. Physiol. Mol. Plant Pathol. **64,** 245-254.

Slade, A.J., Fuerstenberg, S.I., Loeffler, D., Steine, M.N., and Facciotti, D. (2005). A reverse genetic, nontransgenic approach to wheat crop improvement by TILLING. Nat. Biotechnol. **23,** 75-81.

Smit, R., and Tudzynski, P. (1992). Efficient transformation of *Claviceps purpurea* using pyrimidine auxotrophic mutants: cloning of the OMP decerboxylase gene. Mol. Gen. Genet. **234,** 297-305.

Snijders, C.H.A. (1990). Genetic variation for resistance to Fusarium head blight in bread wheat. Euphytica **50,** 171-179.

Snijders, C.H.A. (1994). Breeding for resistance to *Fusarium* in wheat and maize. In Mycotoxins In Grain: Compounds Other Than Aflatoxin, J.D. Miller and H.L. Trenholm, eds (St Paul: Eagan Press), pp. 37-58.

Snijders, C.H.A. (2004). Resistance in wheat to *Fusarium* infection and trichothecene formation. Toxicol. Lett. **153,** 37-46.

Snijders, C.H.A., and Perkowski, J. (1990). Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. Phytopathology **80,** 566-570.

Snijders, C.H.A., and Van Eeuwijk, F.A. (1991). Genotype x strain interactions for resistance to *Fusarium* head blight caused by *Fusarium culmorum* in winter wheat. Theoretical and Applied Genetics **81,** 239-244.

Snijders, C.H.A., and Krechting, C.F. (1992). Inhibition of deoxynivalenol translocation and fungal colonization in Fusarium head blight resistant wheat. Canadian Journal of Botany **70,** 1570-1576.

Solomon, P.S., Lowe, R.G.T., Tan, K.C., Waters, O.D.C., and Oliver, R.P. (2006). *Stagonospora nodorum*: cause of stagonospora nodorum blotch of wheat. Mol. Plant Pathol. **7,** 147-156.

Somers, D.J., Fedak, G., and Savard, M. (2003). Molecular mapping of novel genes controlling *Fusarium* head blight resistance and deoxynivalenol accumulation in spring wheat. Genome **46,** 555-564.

Somers, D.J., Thomas, J., DePauw, R., Fox, S., Humphreys, G., and Fedak, G. (2005). Assembling complex genotypes to resist *Fusarium* in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics **111,** 1623-1631.

Srichumpa, P., Brunner, S., Keller, B., and Yahiaoui, N. (2005). Allelic series of four powdery mildew resistance genes at the *Pm3* locus in hexploid bread wheat. Plant Physiol. **139,** 885-895.

Srinivasachary, Gosman, N., Steed, A., Hollins, T.W., Bayles, R., Jennings, P., and Nicholson, P. (2009). Semi-dwarfing *Rht-B1* and *Rht-D1* loci of wheat differ significantly in their influence on resistance to Fusarium head blight. Theoretical and Applied Genetics **118,** 695-702.

Srinivasachary, Gosman, N., Steed, A., Simmonds, J., Leverington-Waite, M., Wang, Y., Snape, J., and Nicholson, P. (2008). Susceptibility to *Fusarium* head blight is associated with the *Rht-D1b* semi-dwarfing allele in wheat. Theoretical and Applied Genetics **116,** 1145-1153.

Stack, R.W., and McMullen, M.P. (1985). Head blighting potential of *Fusarium* species associated with spring wheat heads. Canadian Journal of Plant Pathology **7,** 79-82.

Stack, R.W., Frohberg, R.C., and Casper, H. (1997). Reaction of spring wheats incorporating Sumai#3-derived resistance to inoculation with seven *Fusarium* species. Cereal Res. Commun. **25,** 667-671.

Starkey, D.E., Ward, T.J., Aoki, T., Gale, L.R., Kistler, H.C., Geiser, D.M., Suga, H., Toth, B., Varga, J., and O'Donnell, K. (2007). Global molecular surveillance reveals novel *Fusarium* head blight species and trichothecene toxin diversity. Fungal Genet. Biol. **44,** 1191-1204.

Stein, N., and Graner, A. (2005). Map-based isolation in cereal genomes. In Cereal Genomics, P.K. Gupta and R.K. Varshney, eds (NOT SURE: Springer), pp. 331-360.

Stein, N., Feuillet, C., Wicker, T., Schalagenhauf, E., and Keller, B. (2000). Subgenome chromosome walking in wheat: a 450-kb physical contig in *Triticum monococcum* L. spans the *Lr10* resistance locus in hexaploid wheat (*Triticum aestivum* L.). Proc. Natl. Acad. Sci. U. S. A. **97,** 13436-13441.

Steiner, B., Lemmens, M., Griesser, M., Scholz, U., Schondelmaier, J., and Buerstmayr, H. (2004). Molecular mapping of resistance to *Fusarium* head blight in the spring wheat cultivar Frontana. Theoretical and Applied Genetics **109,** 215-224.

Stephens, A.E., Gardiner, D.M., White, R.G., Munn, A.L., and Manners, J.M. (2008). Phases of infection and gene expression of *Fusarium graminearum* during crown rot disease of wheat. Molecular Plant Microbe Interactions **21,** 1571-1581.

Strange, R.N., and Smith, H. (1971). A fungal growth stimulant in anthers which predisposes wheat to attack by *Fusarium graminearum*. Physiological Plant Pathology **1,** 141-150.

Strange, R.N., Majer, J.R., and Smith, H. (1974). Isolation and identification of choline and betaine as two major components in anthers and wheat germ that stimulate *Fusarium graminearum in vitro*. Physiological Plant Pathology **4,** 277-290.

Sun, G., Bond, M., Nass, H., Martin, R., and Dong, Z. (2003). RAPD polymorphisms in spring wheat cultivars and lines with different level of Fusarium resistance. Theoretical and Applied Genetics **106,** 1059-1067.

Sutton, J. (1982). Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology **4,** 195-209.

Tacke, B.K., and Casper, H.H. (1996). Determination of deoxynivalenol in wheat, barley, and malt by column cleanup and gas chromatography with electron capture detection. Journal of AOAC International **79,** 472-475.

Tada, S., Gomi, K., Kitamoto, K., Takahashi, K., Tamura, G., and Hara, S. (1991). Construction of a fusion gene comprising the Taka-amylase A promoter and the *Escherichia coli* beta-glucuronidase gene and analysis of its expression in *Aspergillus oryzae*. Mol. Gen. Genet. **229,** 301-306.

Tag, A.G., Garifullina, G.F., Peplow, A.W., Ake, C., Phillips, T.D., Hohn, T.M., and Beremand, M.N. (2001). A novel regulatory gene, *Tri10*,

controls trichothecene toxin production and gene expression. Appl. Environ. Microbiol. **67,** 5294-5302.

TAGI. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature **408,** 796-815.

Tamburic-Ilincic, L., Schaafsma, A.W., Falk, D., Laskar, B., Fedak, G., and Somers, D. (2006). Registration of winter wheat germplasm line RCATL33 with Fusarium head blight resistance and reduced deoxynivalenol accumulation. Crop Science **46,** 1399-1400.

Taylor, R.D., Saparno, A., Blackwell, B., Anoop, V., Gleddie, S., Tinker, N.A., and Harris, L.J. (2008). Proteomic analyses of *Fusarium graminearum* grown under mycotoxin-inducing conditions. Proteomics **8,** 2256-2265.

Teich, A.H., and Hamilton, J.R. (1985). Effect of cultural practices, soil phosphorus, potassium, and pH on the incidence of Fusarium head blight and deoxynivalenol levels in wheat. Appl. Environ. Microbiol. **49,** 1429-1431.

Teich, A.H., Shugar, L., and Smid, A. (1987). Soft white winter wheat cultivar field-resistance to scab and deoxynivalenol accumulation. Cereal Res. Commun. **15,** 109-114.

Tokai, T., Koshino, H., Takahashi-Ando, N., Sato, M., Fujimura, M., and Kimura, M. (2007). *Fusarium Tri4* encodes a key multifunctional cytochrome P450 monooxygenase for four consecutive oxygenation steps in trichothecene biosynthesis. Biochem. Biophys. Res. Commun. **353,** 412-417.

Trail, F. (2009). For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. Plant Physiol. **149,** 103-110.

Trail, F., Gaffoor, I., Guenther, J.C., and Hallen, H.E. (2005). Using genomics to understand the disease cycle of the Fusarium head blight fungus, *Gibberella zeae* (anamorph *Fusarium graminearum*). Canadian Journal of Plant Pathology **27,** 486-498.

Travella, S., Klimm, T.E., and Keller, B. (2006). RNA interference-based gene silencing as an efficient tool for functional genomics in hexaploid bread wheat. Plant Physiol. **142,** 6-20.

Tudzynski, P., and Scheffer, J. (2004). *Claviceps purpurea*: molecular aspects of a unique pathogenic lifestyle. Mol. Plant Pathol. **5,** 377-388.

Urban, M., Daniels, S., Mott, E., and Hammond-Kosack, K. (2002). *Arabidopsis* is susceptible to the cereal ear blight fungal pathogens *Fusarium graminearum* and *Fusarium culmorum*. Plant J. **32,** 961-973.

Urban, M., Mott, E., Farley, T., and Hammond-Kosack, K. (2003). The *Fusarium graminearum MAP1* gene is essential for pathogenicity and development of perithecia. Mol. Plant Pathol. **4,** 347-359.

Utz, H.F., Melchinger, A.E., and Schon, C.C. (2000). Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics **154,** 1839-1849.

Van der Biezen, E.A., and Jones, J.D.G. (1998). Plant disease-resistance proteins and the gene-for-gene concept. Trends in Biochemical Sciences **23,** 454-456.

van der Hoorn, R.A.L., and Kamoun, S. (2008). From guard to decoy: a new model for perception of plant pathogen effectors. Plant Cell **20,** 2009-2017.

Van Eeuwijk, E.A., Mesterhazy, A., Kling, C.I., Ruckenbauer, P., Saur, L., Burstmayr, H., Lemmens, M., Keizer, L.C.P., Maurin, N., and Snijders, C.H.A. (1995). Assessing non-specificity of resistance in wheat to head blight caused by inoculation with European strains of *Fusarium culmorum*, *F. graminearum* and *F. nivale* using a multiplicative model for interaction. Theoretical and Applied Genetics **90,** 221-228.

Van Hemelrijck, W., Wouters, P.F.W., Brouwer, M., Windelinckx, A., Goderis, I.J.W.M., De Bolle, M.F.C., Thomma, B.P.H.J., Cammue, B.P.A., and Delaure, S.L. (2006). The Arabidopsis defense response mutant *esa1* as a model to discover novel resistance traits against *Fusarium* diseases. Plant Sci. **171,** 585-595.

Van Sanford, D., Anderson, J., Campbell, K., Costa, J., Cregan, P., Griffey, C., Hayes, P., and Ward, R. (2001). Discovery and deployment of molecular markers linked to Fusarium head blight resistance: an integrated system for wheat and barley. Crop Science **41,** 638-644.

Van Sanford, D.A., Connelley, J., Swanson, C.S., Kennedy, B., Tutt, C.R., Tomes, L.J., Hershman, D.E., Gaines, C., Jin, Y., Bockelman, H.E., Long, D.L., and Cambron, S.E. (2006). Registration of 'Allegiance' wheat. Crop Science **46,** 2305-2306.

Vanco, B., Slikova, S., and Sudyova, V. (2007). Influence of localities and winter wheat cultivars on deoxynivalenol accumulation and disease damage by *Fusarium culmorum*. Biologia **62,** 62-66.

Varshney, R.K., Balyan, H.S., and Langridge, P. (2006). Wheat. In Genome Mapping and Molecular Breeding in Plants, C. Kole, ed (Berlin: Springer-Verlag), pp. 79-134.

Voigt, C.A., Schafer, W., and Salomon, S. (2005). A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. Plant J. **42,** 364-375.

Voigt, C.A., von Scheidt, B., Gacser, A., Kassner, H., Lieberei, R., Schafer, W., and Salomon, S. (2007). Enhanced mycotoxin production of a lipase-deficient *Fusarium graminearum* mutant correlates to toxin-related gene expression. European Journal of Plant Pathology **117,** 1-12.

Voss, H.-H., Holzapfel, J., Hartl, L., Korzun, V., Rabenstein, F., Ebmeyer, E., Coester, H., Kempf, H., and Miedaner, T. (2008). Effect of the *Rht-D1* dwarfing locus on *Fusarium* head blight rating in three segregating populations of winter wheat. Plant Breeding **127,** 333-339.

Waalwijk, C., van der Heide, R., de Vries, I., van der Lee, T., Schoen, C., Costrel-de Corainville, G., Hauser-Hahn, I., Kastelein, P., Kohl, J., Lonnet, P., Demarquet, T., and Kema, G.H.J. (2004). Quantitative detection of *Fusarium* species in wheat using TaqMan. European Journal of Plant Pathology **110,** 481-494.

Waldron, B.L., Moreno-Sevilla, B., Anderson, J.A., Stack, R.W., and Frohberg, R.C. (1999). RFLP mapping of QTL for Fusarium head blight resistance in wheat. Crop Science **39,** 805-811.

Wang, J.H., Pawelzik, E., Weinert, J., and Wolf, G.A. (2005). Impact of *Fusarium culmorum* on the polysaccharides of wheat flour. J. Agric. Food Chem. **53,** 5818-5823.

Wang, Y.Z., and Miller, J.D. (1988). Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathol.-Phytopathol. Z. **122,** 118-125.

Wanjiru, W.M., Kang, Z.S., and Buchenauer, H. (2002). Importance of cell wall degrading enzymes produced by *Fusarium graminearum* during infection of wheat heads. European Journal of Plant Pathology **108,** 803-810.

Wei, Y.-M., Hou, Y.-C., Yan, Z.-H., Wu, W., Zhang, Z.-Q., Liu, D.-C., and Zheng, Y.-L. (2005). Microsatellite DNA polymorphism divergence in Chinese wheat (*Triticum aestivum* L.) landraces highly resistant to *Fusarium* head blight. Journal of Applied Genetics **46,** 3-9.

Wilcoxson, R.D., Busch, R.H., and Ozmon, E.A. (1992). Fusarium head blight resistance in spring wheat cultivars. Plant Disease **76,** 658-661.

Wilde, F., Schon, C.C., Korzun, V., Ebmeyer, E., Schmolke, M., Hartl, L., and Miedaner, T. (2008). Marker-based introduction of three quantitative-trait loci conferring resistance to *Fusarium* head blight into an independent elite winter wheat breeding population. Theoretical and Applied Genetics **117,** 29-35.

Windels, C.E. (2000). Economic and social impacts of Fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology **90,** 17-21.

Wisser, R.J., Balint-Kurti, P.J., and Nelson, R.J. (2006). The genetic architecture of disease resistance maize: a synthesis of published studies. Phytopathology **96,** 120-129.

Wuchiyama, J., Kimura, M., and Yamaguchi, I. (2000). A trichothecene efflux pump encoded by *Tri102* in the biosynthetic gene cluster of *Fusarium graminearum*. Journal of Antibiotics **53,** 196-200.

Xu, J.R., Peng, Y.L., Dickman, M.B., and Sharon, A. (2006). The dawn of fungal pathogen genomics. Annu. Rev. Phytopathol. **44,** 337-366.

Xu, X.M., Parry, D.W., Nicholson, P., Thomsett, M.A., Simpson, D., Edwards, S.G., Cooke, B.M., Doohan, F.M., Brennan, J.M., Moretti, A., Tocco, G., Mule, G., Hornok, L., Giczey, G., and Tatnell, J. (2005). Predominance and association of pathogenic fungi causing Fusarium ear blight in wheat in four European countries. European Journal of Plant Pathology **112,** 143-154.

Yahiaoui, N., Srichumpa, P., Dudler, R., and Keller, B. (2004). Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. The Plant Journal **37,** 528-538.

Yang, J., Bai, G.H., and Shaner, G.E. (2005a). Novel quantitative trait loci (QTL) for Fusarium head blight resistance in wheat cultivar Chokwang. Theoretical and Applied Genetics **111,** 1571-1579.

Yang, L., van der Lee, T., Yang, X., Yu, D., and Waalwijk, C. (2008). *Fusarium* populations on Chinese barley show a dramatic gradient in mycotoxin profiles. Phytopathology **98,** 719-727.

Yang, Z.P., Gilbert, J., and Procunier, J.D. (2006). Genetic diversity of resistance genes controlling fusarium head blight with simple sequence repeat markers in thirty-six wheat accessions from East Asian origin. Euphytica **148,** 345-352.

Yang, Z.P., Gilbert, J., Fedak, G., and Somers, D.J. (2005b). Genetic characterization of QTL associated with resistance to Fusarium head blight in a doubled-haploid spring wheat population. Genome **48,** 187-196.

Yates, I.E., and Sparks, D. (2008). *Fusarium verticillioides* dissemination among maize ears of field-grown plants. Crop Prot. **27,** 606-613.

Yates, I.E., Hiett, K.L., Kapczynski, D.R., Smart, W., Glenn, A.E., Hinton, D.M., Bacon, C.W., Meinersmann, R., Liu, S., and Jaworski, J. (1999). GUS transformation of the maize fungal endophyte *Fusarium moniliforme*. Mycological Research **103,** 129-136.

Young, N.D. (1996). QTL mapping and quantitative disease resistance in plants. Annu. Rev. Phytopathol. **34,** 479-501.

Yu, G., Ma, H., Xu, Z., Ren, L., Zhou, M., and Lu, W. (2004). Cloning a DNA marker associated to wheat scab resistance. Journal of Applied Genetics **45,** 17-25.

Yu, G.H., Ma, H.X., Bai, G.H., and Tang, K.X. (2008a). Single-strand confromational polymorphism markers associated with a major QTL for Fusarium head blight resistance in wheat. Molecular Biology **42,** 504-513.

Yu, H.Y., Seo, J.A., Kim, J.E., Han, K.H., Shim, W.B., Yun, S.H., and Lee, Y.W. (2008b). Functional analyses of heterodimeric G protein G alpha and G beta subunits in *Gibberella zeae*. Microbiology **154,** 392-401.

Yu, J., Hu, S., Wang, J., Wong, G.S.K., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen, Y., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Huang, X., Li, W., Li, J., Liu, Z., Li, L., Liu, J., Qi, Q., Liu, J., Li, L., Li, T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X., Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He, S., Zhang, J., Xu, J., Zhang, K., Zhang, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Ren, X., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Xia, H., Bao, Q., Li, G., Gao, H., Cao, T., Wang, J., Zhao, W., Li, P., Chen, W., Wang, X., Zhang, Y., Hu, J., Wang, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, G., Zhu, Z., Chen, R., Hao, B., Zheng, W., Chen, S., Guo, W., Li, G., Liu, S., Tao, M., Wang, J., Zhu, L., Yuan, L., and Yang, H. (2002). A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science **296,** 79-92.

Yu, J.B., Bai, G.H., Cai, S.B., and Ban, T. (2006). Marker assisted characterization of Asian wheat lines for resistance to *Fusarium* head blight. Theoretical and Applied Genetics **113,** 308-320.

Yu, J.B., Bai, G.H., Cai, S.B., Dong, Y.H., and Ban, T. (2008c). New Fusarium head blight-resistant sources from Asian wheat germplasm. Crop Science **48,** 1090-1097.

Yu, J.B., Bai, G.H., Zhou, W.C., Dong, Y.H., and Kolb, F.L. (2008d). Quantitative trait loci for Fusarium head blight resistance in a recombinant inbred population of Wangshuibai/Wheaton. Phytopathology **98,** 87-94.

Yu, Y.-J. (1982). Monosomic analysis for scab resistance and yield components in the wheat cultivar Soo-mo 3. Cereal Res. Commun. **10,** 185-189.

Zapf, M.W., Theisen, S., Vogel, R.F., and Niessen, L. (2006). Cloning of wheat LTP1500 and two *Fusarium culmorum* hydrophobins in *Saccharomyces cerevisiae* and assessment of their gushing inducing potential in experimental wort fermentation. J. Inst. Brew. **112,** 237-245.

Zhang, X., Zhou, M.P., Ren, L.J., Bai, G.H., Ma, H.X., Scholten, O.E., Guo, P.G., and Lu, W.Z. (2004). Molecular characterization of *Fusarium* head blight resistance from wheat variety Wangshuibai. Euphytica **139,** 59-64.

Zhou, T., He, J., and Gong, J. (2008). Microbial transformation of trichothecene mycotoxins. World Mycotoxin Journal **1,** 23-30.

Zhou, W.C., Kolb, F.L., Bai, G.H., Domier, L.L., and Yao, J.B. (2002a). Effect of individual Sumai 3 chromosomes on resistance to scab spread within spikes and deoxynivalenol accumulation within kernels in wheat. Hereditas **137,** 81-89.

Zhou, W.C., Kolb, F.L., Bai, G.H., Shaner, G., and Domier, L.L. (2002b). Genetic analysis of scab resistance QTL in wheat with microsatellite and AFLP markers. Genome **45,** 719-727.

Zhou, W.C., Kolb, F.L., Bai, G.H., Domier, L.L., Boze, L.K., and Smith, N.J. (2003). Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. Plant Breeding **122,** 40-46.

Zhou, W.C., Kolb, F.L., Yu, J.B., Bai, G.H., Boze, L.K., and Domier, L.L. (2004). Molecular characterization of *Fusarium* head blight resistance in Wangshuibai with simple sequence repeat and amplified fragment length polymorphism markers. Genome **47,** 1137-1143.

Zipfel, C., and Felix, G. (2005). Plants and animals: a different taste for microbes? Curr. Opin. Plant Biol. **8,** 353-360.

APPENDIX

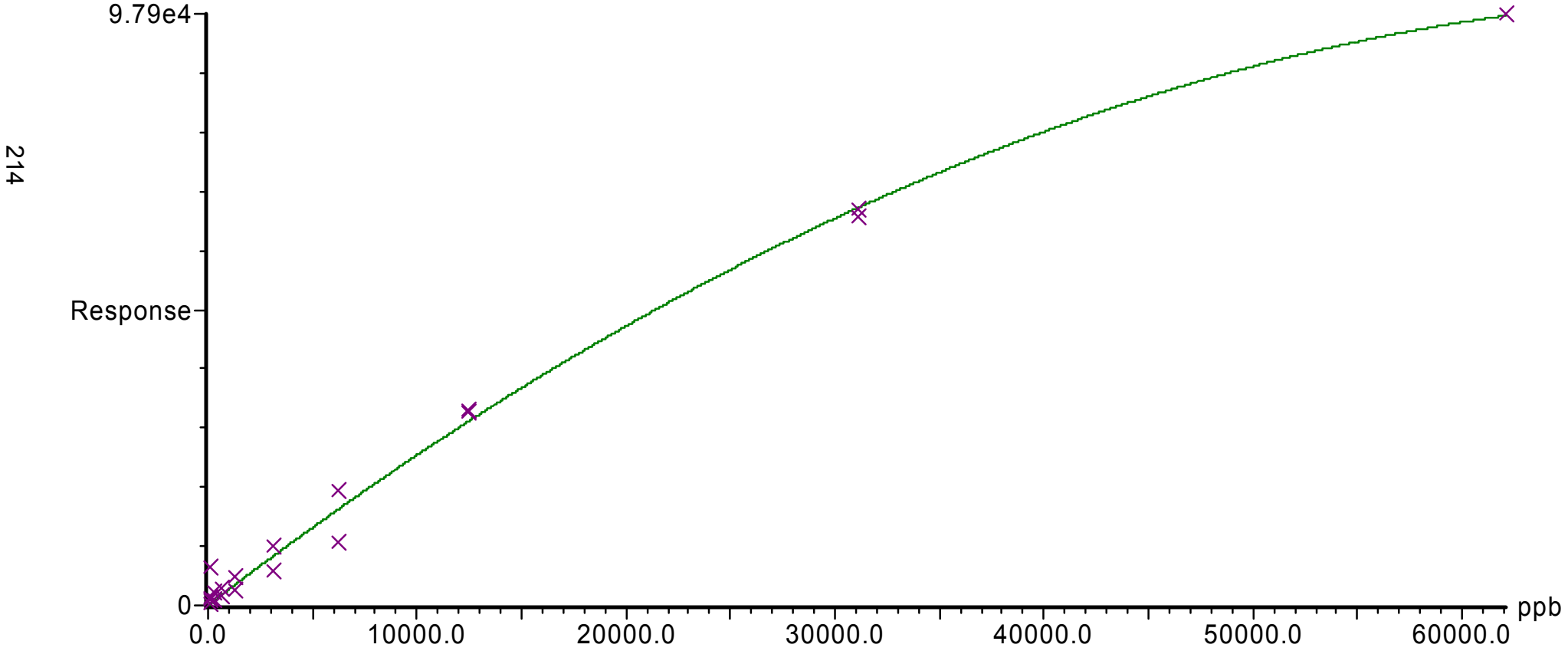
A1 Pedigree information for selected genotypes from the field trials

Genotype	Official name	Commercial release date	Breeding scheme	Breeding location	Grain type /quality	Overall FEB rating China	Known FEB QTLs
China 1	Wanmai-43	2001	RC*1	Hefei	White	R	
China 2	Sumai-3	Breeding line only	Funo/Taiwan wheat	Suzhou	Red	Extreme R	3BS 5A 6B
China 3	Z-9023	2001	(Xiaoyan6×Xinong65□ ×[83□2□3-3×84□14□43])F3×Shan213	Zhenzhou	White / high	Mod R	
China 4	Wanmai-27	1996	Pedigree 1	Hefei	White	R/MR	
China 5	Ningmai-8	1996	Pedigree 2	Nanjing	Red	MR	
China 6	Wan9926	2004	RC*1	Hefei	White	R/MR	
China 7	N962390	2003	TC with HA*** China 8 somatic line	Hefei	Red	Highly R	
China 8	Yang158	1993	Pedigree 3 Nanda2419/Triumph// Funo/3/st1472/506	Yangzhou / Jiangsu province	Red	Mod R	2D 4B
CIMMYT 2	Unknown					Not tested	
USA1	Jagger	1997	KS82W418/'Stephens'	Kansas Agricultural Experiment Station and USDA ARS	Hard red	Not tested	
Bobwhite	Bobwhite			Cimmyt		Not tested	

RC*1 – recurrent selection for yield and high FEB resistance with some parents in common and included Sumai 3
Pedigree 1 - some different parents to China 1 and China 6
Pedigree 2 - some parents in common with China 8
Pedigree 3 – some parents in common with China 5
TC with HA*** - tissue culture on China 8 with haploid apogamy and chemistry to produce China 7
China 8 and China 5 come from two different breeding groups in China but with some parents in common

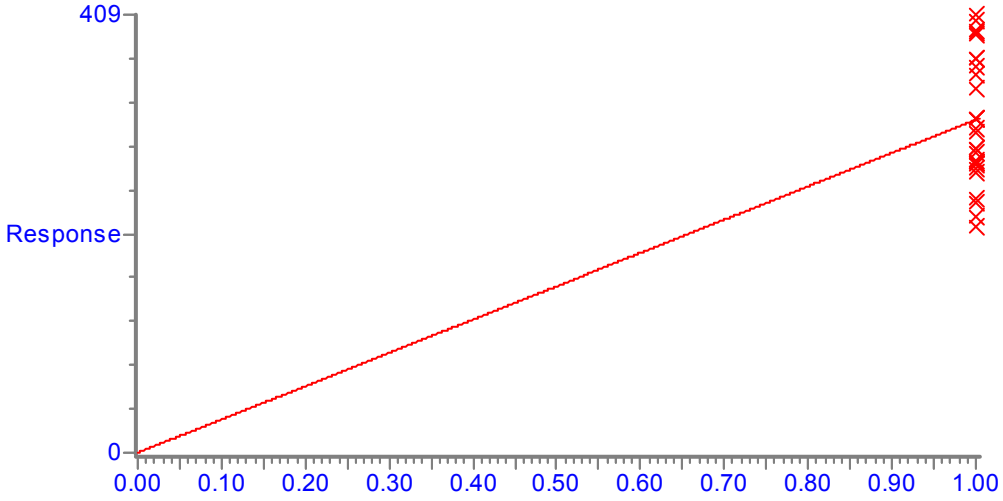
A4 Calibration curve for 2006 analyses (DON)

Compound 2 name: DON (all) Method File: DON 2006
Coefficient of Determination: 0.992488
Calibration curve: -1.75421e-5 * x^2 + 2.66051 * x + 0
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: 2nd Order, Origin: Force, Weighting: Null, Axis trans: None

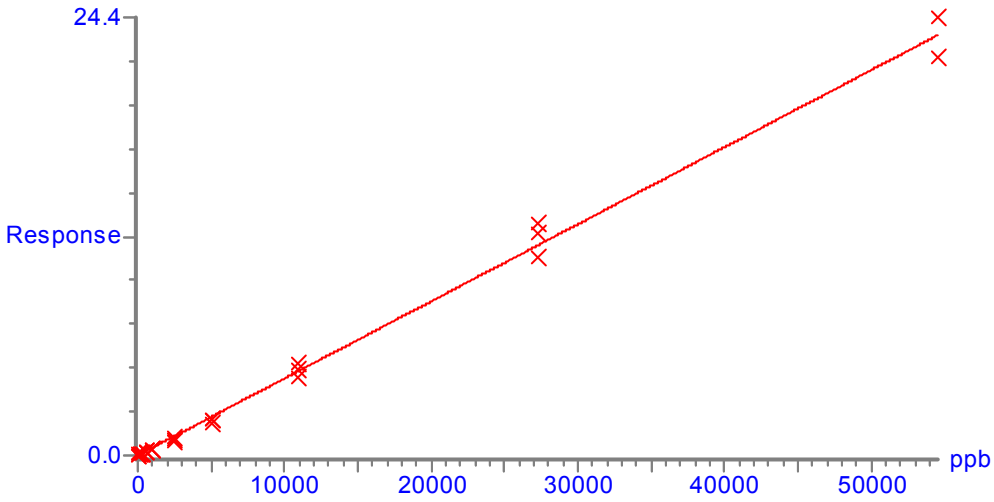


A5 Calibration curves for the 2007 analysis (DOM-1, DON, 3-ADON, 15-ADON, NIV)

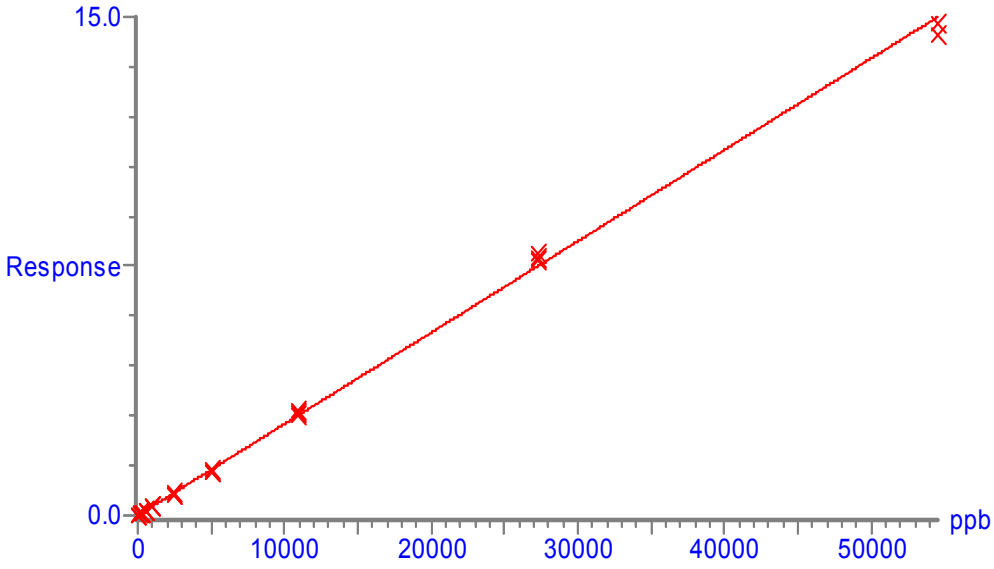
Compound name: DOM-1
Response Factor: 310.728
RRF SD: 57.8426, % Relative SD: 18.6152
Response type: External Std, Area
Curve type: RF



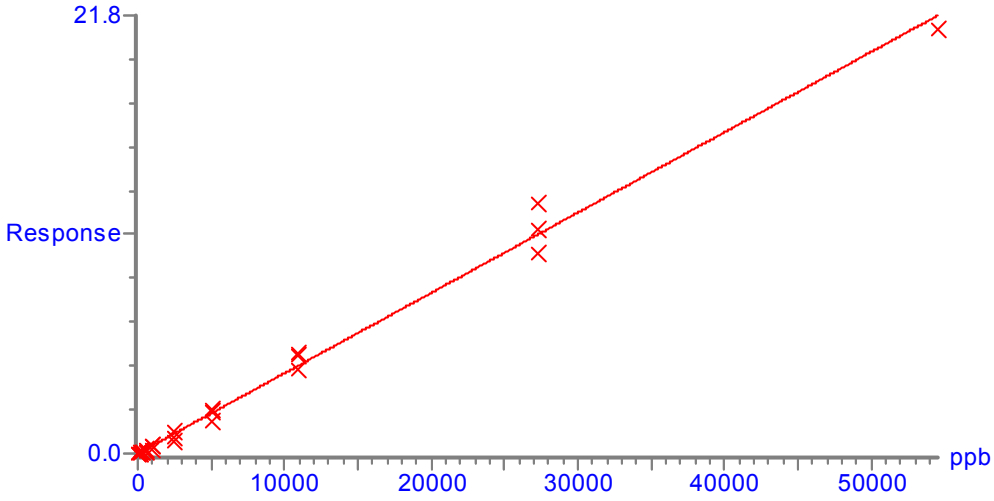
Compound name: DON
Coefficient of Determination: R^2 = 0.992138
Calibration curve: 0.000429292 * x
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Force, Weighting: 1/x, Axis trans: None



Compound name: 3-ADON
Coefficient of Determination: R^2 = 0.997955
Calibration curve: 0.000274747 * x
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Force, Weighting: 1/x, Axis trans: None



Compound name: 15-ADON
Coefficient of Determination: R^2 = 0.987052
Calibration curve: 0.000399863 * x
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Force, Weighting: 1/x, Axis trans: None



Compound name: NIV
Coefficient of Determination: R^2 = 0.759744
Calibration curve: 0.000136557 * x
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Force, Weighting: 1/x, Axis trans: None