

Investigating disease tolerance to *Zymoseptoria tritici* in wheat

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Declaration

I hereby declare that all the work presented in this thesis has been conducted by me and is my own, with the exception of the disease symptom assessments during the season and the growth analyses at GS32 and harvest in the field experiment at ADAS Rosemaund in 2014, for which I was assisted by Julie Smith and colleagues at ADAS Rosemaund.

Abstract

Disease tolerance is defined as the ability to maintain grain yield in the presence of disease and could be a potential defence mechanism to be incorporated into breeding programmes. It is an attractive goal, as disease tolerance has the potential to be a broad-spectrum, durable defence mechanism while exerting little selection pressure on pathogen populations. Relatively little is known about how disease tolerance is conferred, but most of the hypotheses suggest resource capture and resource-use traits such as large green canopy area, increased light extinction coefficient and a high source to sink balance. Disease tolerance in current wheat genotypes is generally associated with low yield potential, and for disease tolerance to be incorporated into commercial breeding it is important to determine whether this link can be disassociated.

In this study, an attempt was made to identify physiological traits conferring disease tolerance to Septoria tritici blotch (STB) in winter wheat. Wheat genotypes contrasting in disease tolerance were selected for in-depth phenotyping of selected physiological traits to determine their association with disease tolerance. A number of publications have attempted to link disease tolerance to physiological traits in wheat, based on their yield loss to disease symptom relationship. However, in this study it was proposed that variation in non-symptomatic disease could influence the appearance of disease tolerance which has not previously been investigated. The ratio of in-leaf pathogen biomass to visual disease symptoms was studied in both controlled-environment experiments and in field experiments to determine whether a high in-leaf pathogen biomass was associated with disease tolerance.

Two field experiments were conducted during the field seasons 2011/12 and 2013/14 at Teagasc Oak Park, Carlow, Ireland and ADAS Rosemaund, Herefordshire, UK, respectively. A field experiment was also conducted in 2012/13 at Teagasc Oak Park, but due to dry conditions and little disease presence this field experiment was excluded from nearly all experimental analyses. In each experiment, there were two fungicide treatments, non-target disease control and full disease control. In order to increase genetic variability, 38 selected lines from a L14 x Rialto doubled-haploid (DH) mapping population developed by the International Maize and

Wheat Improvement Centre (CIMMYT) were screened alongside 10 UK-adapted reference genotypes for contrasting disease tolerance in 2012. Tolerance was quantified as yield loss per unit of green lamina area index (GLAI) loss to disease. L14 is a CIMMYT spring wheat large-ear phenotype advanced line and Rialto is a UK winter wheat which has high radiation-use efficiency and stem soluble carbohydrate. The DH lines displayed an increased range of disease tolerance compared to the UK-adapted reference genotypes.

Selected genotypes were subjected to in-depth phenotyping for an extended range of physiological traits in 2014 to identify traits associated with increased disease tolerance. The traits measured included pre- and post-anthesis radiation interception, light extinction coefficient at anthesis, pre- and post anthesis radiation-use efficiency and stem water soluble carbohydrate accumulation at ear emergence + 7 days. In general, there was a wide range of physiological traits displaying weak associations with disease tolerance. The main traits associated with disease tolerance were related to large and/or maintained source capacity in the presence of disease, such as increased GLAI at anthesis and increased post-anthesis light interception. There was also a general association with low grain yield in the absence of disease and decreased harvest index. Increased disease tolerance was associated with high source capacity and low sink capacity, and there was an association between a high source to sink balance, measured as increased Healthy Area Duration (HAD) per grain, and disease tolerance.

The impact of genotype variation on the amount of non-symptomatic disease to visual disease expression was investigated in controlled-environment (CE) experiments. In-leaf *Zymoseptoria tritici* fungal biomass (pathogen load) was quantified by a Real Time qPCR assay targeting the β-tubulin gene (Accession no. AY547264) and compared to visual disease expression. In the first CE experiment, two wheat genotypes were exposed to increasing concentrations of *Z. tritici* inoculum. There were differences in rates of pathogen development and pathogen presence between inoculum concentrations in both visual disease symptoms and pathogen loads. In the following CE experiment, a wider range of genotypes exposed to a high inoculum level were shown to differ significantly in the relationship between visual disease symptoms and pathogen loads.

In order to determine the impact of genotype variation on the visual disease symptoms to pathogen load ratio, flag leaves of genotypes screened for in-field disease tolerance in 2012 and 2014 were analysed. Large variations in the disease symptoms to pathogen load ratio were identified, which has not previously been shown in wheat experiments. An attempt was made to relate the visual symptoms – pathogen load ratio to non-lesion green area loss as a measure of a potential metabolic cost of increased pathogen pressure, but no such relationship was found. An increased pathogen load per unit visual symptoms did not account for larger yield losses than predicted for a given disease level and there was no direct relationship between symptom expression - pathogen load ratios and disease tolerance.

The consistency of high/low displays of disease tolerance calculated by different disease measures was investigated using three different ways of measuring disease; HAD, area under disease progress curve (AUDPC) and pathogen DNA quantified by qPCR. In general, the two measures of pathogen presence (AUDPC and pathogen load) tended to quantify disease tolerance similarly, while the HAD-based tolerance contrasted. There were also differences in which traits were associated with disease tolerance for the different methods of calculating tolerance; the calculations based on AUDPC and pathogen DNA tended to associate a decreased source capacity to disease tolerance while the HAD-based tolerance indicated an association with increased source capacity. All methods, however, indicated that a low yield potential was associated with disease tolerance.

In conclusion, there was a large range of disease tolerance found in the field experiments compared to previous investigations. The HAD-based disease tolerance seems to be mainly related to a large source capacity and a low sink capacity. However, the genotype ratings of high/low disease tolerance and associated physiological traits seem to vary according to the method of calculating tolerance. There were large differences in the ratio of visual symptoms-pathogen load between genotypes; even though this did not have a direct impact on disease tolerance or yield loss it could potentially be associated with increased metabolic costs.

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Key terminology and acronyms

Abbreviation	Meaning
AGDM	Above-ground dry matter
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve (% disease days)
avr-protein	Avirulence protein
BLAST	Basic Local Alignment Tool
BYDV	Barley yellow dwarf virus
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo; International Maize and Wheat Improvement Center
df	Degrees of freedom
DH	Doubled-haploid
DM	Dry matter
dpi	Days post inoculation
ELISA	Enzyme-linked immunosorbent asssay
FHB	Fusarium Head Blight
FL	Flag leaf
FLA	Flag leaf area
FT	Fungicide treatment
G	Genotype
GAI	Green area index
GE	Gel electrophoresis
GLA	Green leaf area (%)
GLAI	Green leaf area index
GS	Growth stage (Zadok's)
ha	Hectares
HAD	Healthy area duration (GLAI days)
HGCA	Home-grown Cereals Authority
HI	Harvest index
iPL	Integrated pathogen load (pg STB DNA days)
IPM	Integrated pest management
kPAR	Light extinction coefficient
LAI	Leaf lamina area index
LTM	Long term mean
MP	Multiplex
N	Nitrogen
NCBI	National Centre for Biotechnology Information
ND	NanoDrop
PAR	Photosynthetically active radiation
PCR	Polymerase chain reaction
PL	Pathogen load
QoI	Quinone outside inhibitor
qPCR	Quantitative polymerase chain reaction (synonym: Real-Time PCR)
QR	Quantitative resistance

QTL	Quantitative trait loci
R-gene	Resistance gene
RI	Fractional radiation interception
R-protein	Resistance protein
RUE	Radiation use efficiency
SDHI	Succinate dehydrogenase inhibitor
SDW	Specific dry weight
STB	Septoria tritici blotch
t	Tonnes
TGW	Thousand grain weight
tolAUDPC	Disease tolerance calculated by AUDPC
tolHAD	Disease tolerance calculated by HAD
toliPL	Disease tolerance calculated by iPL
WSC	Water soluble carbohydrates
Y	Year

1. Introduction

1.1 Wheat production

Wheat (*Triticum aestivum L.)* is one of the three main food crops in the world, along with maize and rice. In 2011, wheat was grown on approximately 220 million hectares, yielding over 700 million tonnes of grain worldwide (FAOSTAT, 2013). Wheat has been cultivated for 10,000 years, originating as diploids (einkorn) and tetraploids (emmer) in south eastern Turkey (Shewry, 2009). Today, the modern hexaploid bread wheat is a staple food in a major part of the world, providing 20% of calories for the world population, and is an important source of protein in developing countries (Shewry, 2009, Reynolds et al., 2012). Wheat is also of economic importance in other areas than human food production; in particular, as livestock feed, approximately 45% of wheat production in EU is used for this purpose (FAO, 2002), and to some extent for bioethanol production. Currently wheat grain is used for biofuel production but is also an attractive future use for wheat straw, which can be used for animal bedding but is often discarded (Deswarte et al., 2006, Naik et al., 2010).

Concerns are increasing that the rapidly growing world population will lead to a food crisis (Ray et al., 2013). It has been estimated that the world population will increase to 9.6 billion by 2050 (United Nations, 2013). Furthermore, 870 million people in the world are currently suffering from undernourishment, mostly in developing countries (FAO, 2012). In light of this, food production will have to increase accordingly. Tilman et al. (2011) estimated that global crop production needs to increase by 100-110% between 2005 and 2050, and Hall and Richards (2013) estimated that a yearly increase of between 1.16 and 1.31% is needed to satisfy future global crop demands. For wheat in particular, the annual production will have to increase from the current 700 million tonnes to approx. 850 million tonnes by 2025 (Murchie et al., 2009). Hence, an increase in grain yield per unit area is needed, while doing so in an environmentally sustainable way. In addition to the increase in global population, concern for global food security is increasingly due to future climate change. Different areas will be affected differently, but it is generally accepted that parts of the world where predominantly developing countries are located will be affected the most (IPCC, 2014).

Wheat yields have been increasing nearly linearly over the last 50 years due to a number of changes in crop management and genetic material, but there is some concern that yields have stagnated (Tilman et al., 2011). The average yield in the world was 3.3 t ha^{-1} in 2013 (Fig 1.1), but there are large variations between countries and continents. The highest yields are found in NW Europe, in particular Ireland and Belgium (9.0 and 8.9 t ha^{-1}, respectively in 2013), with the exception of New Zealand that produced average yields of 9.1 t ha^{-1} in 2013. The lowest yields are, however, generally found in developing countries, for instance 0.6 t ha^{-1} in Venezuela and 0.4 t ha^{-1} in Somalia (FAOSTAT, 2014). There is scope for yield improvement in developing countries, in particularly through genetic improvements in resource -use efficiencies such as water and nitrogen, but there is also potential for yield improvements in high output systems such as the UK (Reynolds et al., 2009).

1.2 Plant disease and fungicide dependence

Plant disease is one of the major threats to food security, and affects either the quantity or quality of produced yield. Nearly all food crops are affected by some type of disease, most commonly viruses, bacteria, fungi and oomycetes. Historically, one of the most devastating effects of plant disease was the great Irish potato famine in 1845 during which the population of Ireland was reduced by 2 million due to starvation and mass-emigration (Ó Gráda, 2004). Plant disease remains a major problem despite effective modern fungicides. It has been estimated that plant disease causes between 5 – 15% of word-wide wheat yield losses (Oerke, 2006). The highest percentage of crop losses is in developing countries, for instance, in Central Africa (up to 35%), partly due to a lack of fungicide availability (Oerke, 2006). Crop losses are slightly lower in the high input-high output crop systems in Europe where the use of fungicides is nearly ubiquitous. Despite the effectiveness of fungicide programmes, an estimated 6-7% of wheat grain yield (valued to about £67.5 M in 1998) is lost to plant disease in England and Wales each year (Hardwick et al., 2001).

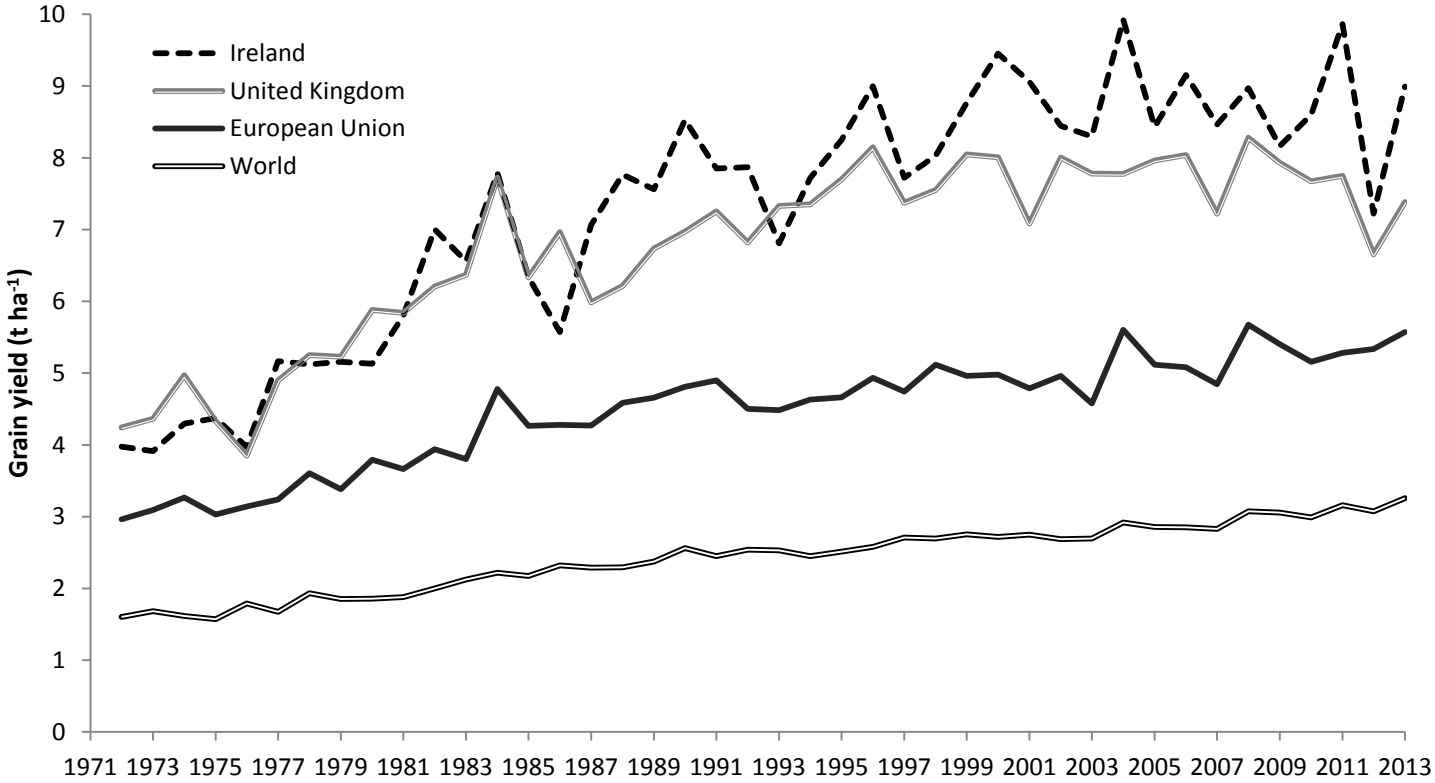


Fig 1.1. The average wheat grain yields between 1972 and 2013 in Ireland, UK, the European Union area and worldwide (FAOSTAT, 2016).

In north-western Europe growers are dependent on fungicides for economically sustainable wheat production, and in the UK fungicide treatment increased from 14% to 91% of wheat crops between 1976 and 1988 (Hardwick et al., 2001). The majority of these fungicides are used to control *Zymoseptoria tritici,* the causal agent of Septoria tritici blotch (STB), which is currently considered to be the most important wheat disease in this part of Europe. This pathogen can cause large yield losses (up to 50%) if not properly managed (Burke and Dunne, 2008), and was calculated to account for £35M of economic losses in England every year (Hardwick et al., 2001). The high fungicide requirement for this pathogen can be problematic, not only as an added expense in strained farm economies, but also due to the development of fungicide insensitivity. A number of fungicides have been rendered ineffective by genetic changes in *Z. tritici* pathogen populations. For instance, the quinone outside inhibitors (QoI) have been overcome by Septoria tritici blotch (Fraaije et al., 2005) and there is increasing fungicide resistance to the azole fungicides (Lucas, 2006, Orton et al., 2011). Recently, *Z. tritici* isolates showing resistance to the newest fungicide technology, the Succinate dehydrogenase inhibitors (SDHI's), have been discovered in Ireland and UK (Dooley et al., 2016b). This is problematic as recent European legislature will complicate market releases of new fungicide chemistries and in addition a number of current fungicides will be withdrawn (Blake et al., 2011). The future of plant protection is therefore likely to be dependent on integrating non-fungicide solutions into crop production systems.

1.3 Options for reduced fungicide dependence

The integration of sustainable plant protection methodologies into crop production systems is called integrated pest management (IPM), and is currently being adopted in EU countries. The basis of IPM is to use environmentally friendly plant protection methods, such as crop rotations, in combination with effective fungicide spray programmes (Shaw and Royle, 1993, van den Berg et al., 2013). Another central premise of IPM is the selection of disease resistant varieties (FAO, 2015). In the adoption of IPM (and restrictions on fungicides), crop self-defence is likely to be a major decision factor.

There are three types of self-defences; disease resistance, disease escape and disease tolerance (Paveley et al., 2005). Disease resistance and escape are based on reduced infection by actively deterring pathogen infection on a cellular level (resistance), or pathogen avoidance by increased height or growing out of synchrony with pathogen development (escape). In contrast, disease-tolerant plants are susceptible to disease infection but able to maintain harvested yield per unit area of disease symptom expression (Bingham et al., 2009).

Disease tolerance could potentially confer durable and broad-spectrum defence against disease. Firstly, genetic improvement to minimise yield loss per unit disease symptom is an attractive goal as it exerts little or no selection pressure on pathogen populations (Bingham et al., 2009). Secondly, disease tolerance has been proposed as a broad-spectrum defence as certain disease-tolerance mechanisms have potential to be effective against a range of pathogen species. This could mean that changes in the spectrum of important pathogens that are likely to occur over time (as the climate and farm practice change) would not result in loss of effect.

Selecting for disease tolerance could form a useful component of durable disease management programmes, especially when used in combination with disease resistance and an effective fungicide spray programme. However, progress towards this end requires a thorough understanding of the phenotypic traits that influence the response of yield to disease, their genetic control, and the possible trade-offs involved with other desirable agronomic characteristics.

1.4 Justification and objectives

The overall aim of this project is to quantify accurately and explain the *Z. tritici* - yield loss relationship (i.e. disease tolerance). Although *Z. tritici* will generally cause yield loss, the actual relationships tend to vary between genotypes and are not fully explained by loss of green area.

One of the main objectives of this project is to determine whether genotype differences in non-symptomatic disease explain the apparent differences in disease symptom – yield loss relationships. Yield loss might not be due solely to loss of light interception through green area loss, but also due to metabolic costs of the plant fighting invisible disease. Little is known about the quantitative effect of pathogen load on grain yield in wheat crops. There is some evidence of differences in in-leaf pathogen biomass (pathogen load) between wheat genotypes in controlled environment studies, but few studies have been carried out on field wheat crops and related to grain yield. In this project, the ratio of in-leaf pathogen load to visual disease expression will be determined and in turn related to yield loss.

The second main objective is to quantify the variation in disease tolerance in UK-adapted winter wheat and exotic wheat germplasm. Due to overall limited genetic diversity in UK-adapted wheat, identifying novel traits and finding extremes in phenotypic traits can be difficult. For this reason it can be an advantage to introduce exotic material in order to widen the genetic diversity. In this project, both UK-adapted wheat and CIMMYT-derived wheat will be investigated in order to identify whether tolerance is associated with the same physiological traits and if the CIMMYT germplasm is a suitable source of novel disease-tolerance traits.

The main hypotheses of this project are:

1. There will be differences in visual disease symptoms, grain yield, and disease tolerance between the genotypes used in the field experiments.
2. There will be differences in disease tolerance between UK adapted winter wheat and wider material derived from crosses with CIMMYT spring wheats associated with novel expression of physiological traits in the CIMMYT germplasm.

3. There will be differences in both quantified pathogen load and pathogen load – visual symptom ratios between genotypes in controlled environments and field experiments.
4. Non-visible disease will have an impact on grain yield and increased ratio of pathogen load to visible disease will cause increased yield losses.

2. Literature review

2.1 Wheat physiology

2.1.1 Crop development and yield formation

Crop formation is dependent on the interception of solar radiation and the conversion of intercepted light to biomass and yield. In general, grain yield potential can be divided into three components according to equation 2.1. The first component, intercepted radiation (RI) is the fraction of available solar radiation intercepted by green photosynthetic tissues. In turn, the efficiency of the conversion of intercepted light into above-ground biomass depends on the radiation-use efficiency (RUE) of the crop. The third factor is the harvest index (HI) of the crop, the proportion of above-ground biomass allocated to the grain.

Eq. 2.1 $$Yield\ potential = RI \times RUE \times HI$$

Only wavelengths of solar radiation in the range 400 – 700 nm can be utilised by leaf tissues and this is commonly referred to as photosynthetically active radiation (PAR). The amount of PAR intercepted depends on the green area index (GAI) and the extinction coefficient (k_{PAR}) of the crop (Mullan and Pietragalla, 2011). The green area index is the ratio of units of green vegetation to the units of ground area. GAI determines how much light is intercepted before it reaches the ground; a higher GAI will intercept more light. The light extinction coefficient (k_{PAR}) of the crop is primarily a measure of the canopy leaf posture. In general, a crop with mainly horizontal leaves will intercept more light in the upper canopy than a crop with erect leaves (Mullan and Pietragalla, 2011, Pask and Pietragalla, 2011).

RUE is defined as the above-ground biomass produced per unit of intercepted light (Stöckle and Kemanian, 2009). The biomass produced by photosynthesis is converted into either structural carbohydrates or water soluble carbohydrates (WSC) that are stored in the stem and other tissues. WSC can be transferred into the grain during late grain-filling, acting as a buffer against post-anthesis stress (Blum, 1998). The proportion of photosynthetic assimilates used for vegetative and yield components differs throughout the crop life cycle, which can be generally divided into the

foundation period, construction period and yield formation period (Sylvester-Bradley et al., 2002)

The foundation period begins at germination and continues until the end of tillering and start of stem extension. During this period, crop resources are invested in vegetative growth by establishing ground cover and producing tillers. In the winter and early spring, light intensity and temperatures are low and as a consequence the relative growth rate of the crop is lower than during the warmer months (Whaley, 2001). The main importance of this period is to establish an optimal number of plants m^{-2} and tillers $plant^{-1}$, which partly determine ears m^{-2} depending on the proportion of tiller survival (Slafer and Whitechurch, 2001).

The construction period is initiated at the beginning of stem extension and initially during this phase crop resources are focused on constructing the stem, the final leaves and the ear. The green area index rapidly increases as the canopy expands and the crop reaches maximum GAI and maximum light interception shortly before flowering. More photosynthate than is needed for structural construction is produced, and this excess is stored in the stem as water soluble carbohydrates (Schnyder, 1993). Numerous studies indicate that this period is of importance for yield formation; particularly in favourable environments where grain growth is sink limited and yield potential is largely determined by grains m^{-2} (Fischer, 2001, Slafer, 2003). It has also been shown that an increased duration of the stem-elongation phase can increase the number of fertile florets per ear through increased assimilate supply to the ear (Miralles et al., 2000).

The grain-filling period starts mid-flowering and lasts until physiological maturity. For the first week post-anthesis, photosynthetic assimilates are allocated to stem WSC and grain. About ten days after flowering, the maximum WSC storage is reached, and in addition to all available photosynthates being used for grain-filling, the WSC stored in the stem are redistributed into the grain (Schnyder, 1993). The duration of the grain-filling period is directly related to yield, particularly so for wet, dull climates such as Ireland and UK (Beed et al., 2007). It has been shown that increasing the duration of the active grain-filling period (by maintaining flag-leaf green area) will increase grain weight and as a result final grain yield (Gooding et al., 2000). Hence, the duration of this period is largely dependent on the

maintenance of green leaf area (and hence photosynthetic activity) in the upper canopy (Paveley et al., 2012), which is primarily restricted by disease during this period.

2.1.2 Quantitative grain yield

The primary objective in wheat production is optimising grain yields, in both quantity and quality. This is of importance, not only for economical profit (ensuring a living for growers) but also for food security reasons as discussed in the introduction. There are several definitions of potential and realised grain yields used in literature:

- Theoretical yield: the maximum yield that could be obtained when all factors are optimised to their theoretical maximum capacity in a given environment.
- Potential yield: Maximum yield that could be obtained with the latest varieties, disregarding environmental factors.
- Attainable yield: When taking environmental factors into account, the attainable yield that could be reached by skilful farmers with optimised crop management (van Ittersum and Rabbinge, 1997).
- Actual yield: the on-farm average yield achieved by the most common crop management practices (van Ittersum et al., 2013).

Some of these definitions will differ according to the author, for instance (Fischer et al., 2009) refer to 'actual yields' as 'average farm yields', and Evans and Fischer (1999) differentiated between "potential yield" (theoretical yield above) and "yield potential" (potential yield above).

There are often considerable differences between potential, attainable and actual yields. Although yields close to the theoretical maximum are reported, the national averages are much lower. In the early 1980's, Gales (1983) reported a maximum potential wheat yield of approximately 12 t ha^{-1} in Britain and a national average of about 50% of this number. Although the maximum potential yields have increased since, estimated to be about 14 t ha^{-1} for a water limited UK phenotype (Sylvester-Bradley et al., 2005), the national average yields are still around 60% of this number. Estimations of the yield gap vary between studies however, for instance Fischer and Edmeades (2010) reported a 30 % gap between potential yield and farm yields in the UK. However, it is not always economically viable for all farmers to attempt to reach the attainable yield level. As yield-input relationships follow

the law of diminishing returns, the cost increase of inputs could be higher than the return in grain yield (van Ittersum et al., 2013).

Yield can be expressed as a product of grains m^{-2} and grain weight (Fig 2.1). Grains m^{-2} is determined by the number of fertile tillers (or ears) m^{-2} and the number of grains produced per ear (Slafer et al., 1996). The number of ear-producing tillers is dependent on a number of factors; cultivar selection such as genotype and winter vs. spring wheat, and by management factors like sowing date and rate, etc. (Frederick and Bauer, 1999). The numbers of grains per ear is determined by the number of spikelets per ear and the number of fertile florets (or grains) per spikelet (Slafer et al., 1996). These are in turn determined by the amount of initiated spikelets and florets, as well as the reduction/abortion of these (Ewert, 1996, González et al., 2011). These factors have been shown to be highly variable due to both biotic and abiotic factors, such as temperature and ear disease. The majority of literature suggests that grains m^{-2} is the major yield determinant in wheat under favourable conditions and much focus has been placed on increasing yields through improving pre-anthesis traits conferring increased grains m^{-2} (Slafer et al., 1996, Fischer, 2001). However, these investigations are predominately based on high radiation environments and might not accurately reflect the conditions in dull, wet climates such as NW Europe. Beed et al. (2007) found that in light-limited, humid climates such as the UK, grain weight played a major part in yield determination. Grain weight is primarily determined by post-anthesis assimilate availability, and therefore directly influenced by the duration of the grain-filling period (Xie et al., 2015).

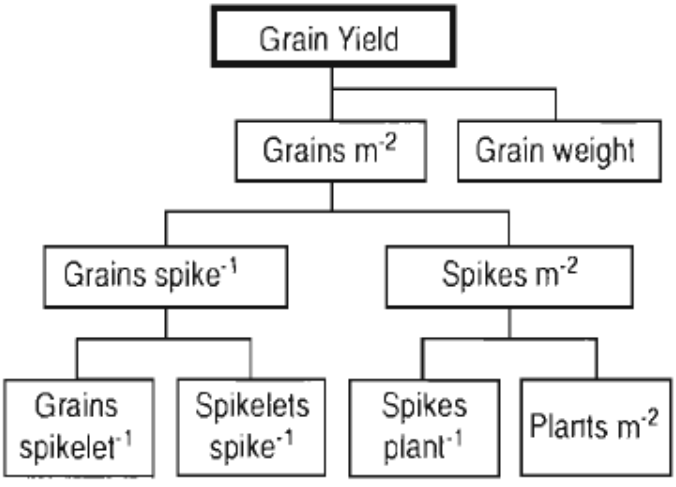


Fig 2.1. Numerical yield determinants, from Slafer et al. (1996).

2.1.3 Wheat breeding in the last century

Wheat yields have been increasing at a steady rate during the last century. In the UK, winter wheat yields have increased at a rate of approximately 100 kg ha^{-1} $year^{-1}$ from 1972 to 2005 (Shearman et al., 2005). These improvements are due to yield potential of the germplasm over time (Slafer et al., 1996). The shift in farming practises as they evolved demanded changes in physiological traits of the wheat cultivars. For instance, as inorganic N fertiliser became more available and cheaper, it was applied at higher rates. As increased amounts of nitrogen were acquired by the crop, canopy green area and duration increased, and consequently increased crop photosynthesis. The prolonged season led to an increase in grain-filling and increased yields, which in turn made the wheat prone to lodging. Due to this; the tall cultivars made way for varieties with short, thick stems that were more lodging resistant. The height reduction in wheat was primarily due to the introduction of the *Rht* semi-dwarfing genes from the Japanese cultivar Norin-50 into CIMMYT breeding programmes during the 1960's (Gale and Youssefian, 1985, Ellis et al., 2002). The introduction of the semi-dwarfing genes *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) improved yields by increasing dry matter allocation to the ear before flowering, leading to increased grain numbers per ear (Shearman et al., 2005). The semi-dwarfing genes are now nearly ubiquitous in wheat grown in NW Europe.

There have been several other genetic changes benefitting yield potential; for instance the 1BL.1RS wheat-rye translocation that was introduced to UK cultivars in the 1980's, in which the wheat 1BL chromosome arm was replaced by the rye 1RS arm (Foulkes et al., 2006). Genotypes containing the 1BL.1RS translocation have been shown to have increased yields (Mujeeb-Kazi et al., 1996). The 7DL.7Ag translocation from *Lophopyrum elongatum* (tall wheat grass, a wild relative of wheat), carrying a rust resistance gene, has been shown to improve grains m^{-2} and biomass accumulation under optimal conditions but conferred lower yields under water-limited conditions (Monneveux et al., 2003). There have also been changes in several phenotypic wheat traits in recent times, such as the selection against awns (Foulkes et al., 2006), decreases in flag leaf area, increased leaf specific weight (Shearman et al., 2005), and a more erect flag leaf position (for improved radiation-use efficiency) is becoming increasingly common (Reynolds et al., 2009).

In general, much of the yield increase since the green revolution was due to increased harvest index (HI), i.e. a higher proportion of the total above ground dry matter being allocated to grain yield compared to structural components. Modern breeding increased harvest index to a current range of 0.5 - 0.6. However, there is an upper limit to how much dry matter can be allocated to the grain while keeping structural components intact – estimated to 0.62 by Austin (1980). Several wheat varieties have demonstrated harvest indices close to this (Mayes et al., 2005). Due to the limited ability of further increase in harvest index, future yield increases might increasingly come from increased biomass – while maintaining a high HI. Investigations of genetic progress in historical cultivars suggest that there has been little change in biomass since the start of the green revolution (Austin et al., 1980, Sayre et al., 1997). However, more recent studies have seen trends in increased biomass over the last two decades (Shearman et al., 2005, Aisawi et al., 2015). A recent study also shows biomass increases in UK bread-making wheat genotypes over the last decade, but not in feed wheat genotypes (Clarke et al., 2012).

2.1.4 Abiotic and biotic factors limiting wheat yields

There are a number of abiotic and biotic factors currently limiting wheat yields. The main abiotic stress affecting wheat production is water limitation, especially with the predicted impacts of climate change. It has been shown that wheat in water-limited areas, such as Australia, China, North American Great Plains and the Mediterranean basin, yield about 40% of their yield potential (Anderson and Angus, 2011). Water deficit has been mentioned as a yield limitation in the UK as well, estimated to cause 10% annual production loss, as about 30% of the UK is drought prone (Dodd et al., 2011). Wheat is also sensitive to extreme temperatures; wheat adapted to cool climates will lose photosynthetic activity at a leaf temperature of 25°C or above and is most sensitive to heat stress (> 30^{o}C) between late booting and flowering (Acevedo et al., 2002). Temperatures above 30°C during this period may reduce grain numbers, and heat stress during flowering may lead to reduced fertility (Farooq et al., 2011). Cold stress, on the other hand, decreases photosynthetic activity and prolonged exposure to cold could lead to photo inhibition and tissue necrosis (Acevedo et al., 2002). Other abiotic stresses include saline toxicity in irrigated systems, and nitrogen deficiency in developing countries (Witcombe et al., 2008).

Biotic stresses that affect wheat are mainly diseases, pests and weeds. The biggest yield-limiting stress in cool, wet, high-yielding climates such as NW Europe is foliar disease. For instance, the fungal disease Septoria tritici blotch has been shown to cause around 4 t ha^{-1} yield losses in Ireland if left untreated (Burke et al., 2011). Oerke and Dehne (2004) estimated that bacterial and fungal pathogens caused worldwide wheat yield losses of 16%. The actual yield losses associated with wheat pathogens were slightly lower at 10% in Western Europe, largely because of successful crop protection (Oerke and Dehne, 2004).

Wheat farmers are today reliant on herbicides for weed control, although farming practices such as ploughing instead of min- or no-till and adjusted sowing dates can reduce weed problems (Cannell and Hawes, 1994, Knezevic et al., 2002). It is imperative to control weeds in wheat fields, as wheat has lost much of its competitiveness over the years. In fact, modern wheat would not be able to survive as a species without cultivation (Shewry, 2009). Weeds are considered to be the main biotic stress when looking at worldwide wheat production, accounting for 23% of actual losses. In Western Europe, the losses caused by weeds were slightly lower at around 9% (Oerke and Dehne, 2004).

2.2 Wheat disease

2.2.1 Measuring plant disease

One of the central premises of studying plant disease, and in particular the plant disease – yield relationships, is to quantify disease accurately. There are three main definitions when quantifying plant pathogens; disease prevalence, disease incidence, and disease severity (Nutter et al., 2006). Disease prevalence and disease incidence are both based on the presence of disease, while disease severity is a measure of the amount of disease (Gaunt, 1995). Below are detailed definitions of these as defined by Nutter et al. (2006):

1. *Disease prevalence* is a term used to describe geographical distribution of a disease. Each location that a disease has been observed, for instance fields, farms, or geographical region such as counties or countries, are expressed as a percentage of total geographic locations.
2. *Disease incidence* is used to describe the number of sampling units, for instance leaves or ears, displaying disease symptoms as a proportion of total leaves that were assessed.
3. *Disease severity* is defined as the amount of disease present on a sampling unit, for instance a leaf or ear. It can be expressed a number of ways; as a percentage of total area, number of lesions/pustules per area, or actual diseased area.

The choice between above methods is dependent on the type of disease and the objective of the study. Disease prevalence is often used in epidemiology studies to map spread of disease. Disease incidence is suitable for identifying early epidemics of nearly all diseases, but primarily for diseases for which presence affects the entire plant or renders it unsuitable for its intended use (Kranz, 1988). Disease severity is the most common measurement in plant pathology studies (Nutter et al., 2006), and will be discussed for the remainder of this section.

There are several methods to assess disease severity. The most common approach is to assess disease visually as a percentage, often with the use of a key (Cooke, 2006). Visual disease assessment has the advantage that no equipment is needed, and it is relatively fast and can be done in large scale studies. However, visual disease assessments have been

shown to be inaccurate and biased by the observer, and less accurate than unbiased methods regardless of which visual assessment method used (Cooke, 2006, Chiang et al., 2015). Studies have found that the largest overestimation of disease occurs at levels below 10% (Parker et al., 1995) or below 25% (Forbes and Jeger, 1987), although there were observation errors at all levels (Forbes and Jeger, 1987). Parker et al. (1995) noted that a biased disease assessment would not be problematic if constant between observers, but this was not the case in their study. Chiang et al. (2015) found a larger effect of observer bias than the visual assessment method on accurate hypothesis testing. Visual assessments of disease symptoms are often used by plant pathologists. When the objective is to examine crop yield losses due to disease, additional measures such as green area duration and radiation interception should be measured as both are directly related to crop yield components (Gaunt, 1995).

More recently, imaging has become an alternative to visual disease assessments. Imaging can include tools such as video cameras, digital cameras, and flat – and slide bed scanners (Bock et al., 2010). Analysis of disease symptoms using imaging software has been shown to be highly accurate (Wijekoon et al., 2008, Bock et al., 2010, Stewart and McDonald, 2014, Mutka and Bart, 2015), particularly on diseases with distinct symptoms. However, it is less useful on diseases with subtle symptoms (Mutka and Bart, 2015). Another recently developed method is disease identification using spectral imaging, a method used in cropping systems for determining crop health and certain physiological traits (Fu et al., 2014, Lee et al., 2010, Kipp et al., 2014). Some studies have identified and quantified disease using spectral indices but this method is still in its early stages of development (Mahlein et al., 2013, Yuan et al., 2014).

The methods described above are based on disease symptoms expressed by the plant. A major drawback of assessing disease symptoms, even if correctly observed, is that they might not necessarily reflect the actual pathogen biomass within a leaf, leading to inaccurate estimations of disease-yield loss relationships (Cooke, 2006). There are two main methods of assessing pathogen load *in planta*; histological and molecular. Histological assessments are based on microscopy observations, using staining or fluorescent markers. Due to the time consuming nature of preparing leaves or other tissues for microscopy, these studies are often small scale. However,

many studies on disease resistance and *in planta* disease development have successfully used these techniques, for instance, Cohen and Eyal (1993) and Metcalfe et al. (2000) used light microscopy staining to study *Zymoseptoria tritici* development and growth in leaves, and Ayliffe et al. (2013) developed a method based on fluorescent markers bound to wheat germ agglutinin to assess disease severity of stem rust.

Molecular disease assessments are becoming increasingly popular. ELISA (enzyme-linked immunosorbent assay) is a useful technique that was initially developed to detect plant viruses (Clark, 1981). Although there are some issues with false positives from interferences of host carbohydrates it is fast, easy and in-field assays have been developed (Fox and Narra, 2006). ELISA assays are however being increasingly replaced by PCR (Polymerase Chain Reaction). PCR can be used for either qualitative or quantitative studies and in particular quantitative PCR (qPCR, also called Real-Time PCR) is of much use in plant disease studies and is becoming more common (Sankaran et al., 2010). Quantitative PCR is a sensitive method with the ability to detect pathogens in a number of materials, from leaf tissue to seed, roots and soil (Schena et al., 2004). It is also a method that has the potential for high-throughput analysis, which is an advantage in large-scale replicated studies. One of the major advantages of quantitative PCR is the ability to detect pre-symptomatic disease and it is therefore an excellent tool in disease prevention**.** Currently a large number of assays have been published for a wide range of cereal pathogens, for instance a number of *Fusarium* species, barley yellow dwarf virus and *Ramularia collo-cygni* (Mumford et al., 2004, Nicolaisen et al., 2009, Taylor et al., 2010). As with any new method, the quality controls reported in early quantitative PCR papers were variable, but guidelines for reporting qPCR-based experiments have now been published (Bustin et al., 2009).

2.2.2 Diseases affecting wheat

There are a number of diseases affecting wheat at different developmental stages and plant organs. Seed-borne fungal diseases cause poor plant-establishment and consequently reduced yields, for instance, *Microdochium nivale* and *Fusarium culmorum* (Hare et al., 1999). These diseases are mainly controlled by seed dressing. A seed-transmitted disease that does not impact the plant until ear formation is ergot (*Claviceps purpurea*), which replaces a grain in the ear with a black sclerotium. Although

causing little yield loss, the sclerotia contain alkaloids which are highly toxic and can cause rejection of grain. There are currently no fully effective fungicides available to treat ergot (Menzies and Turkington, 2014).

Foliar pathogens affect grain yield by reducing photosynthetic green area. The loss of post-anthesis green area has been shown to have a near linear relationship with yield loss, as it affects the production of assimilates during grain-filling and consequently yield (Audsley et al., 2005). In north west Europe, the most severe foliar disease of wheat is septoria tritici blotch (STB), caused by the fungus *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*), which is discussed in detail further below. There are a number of other foliar fungal pathogens affecting wheat yields, such as yellow (stripe) rust (*Puccinia striiformis)* which is common in the east of the UK and can cause major yield loss if left untreated (Wellings, 2011). Two other fungal pathogens targeting the leaf lamina are tan spot (*Pyrenophora tritici-repentis*) and powdery mildew (*Blumeria graminis)* (Lipps and Madden, 1989, Bhathal et al., 2003). A non-fungal disease of some importance is Barley Yellow Dwarf Virus (BYDV), which causes crop stunting, leaf yellowing and deformation (Hoffman and Kolb, 1998). BYDV is transmitted by aphids and therefore not managed by fungicides, but instead by aphid control using insecticides (Gray et al., 1996).

A number of fungal pathogens target the ear, either affecting yield directly by reducing ability of the grain to assimilate carbohydrates or rendering it unsuitable for human or animal consumption. The most economically important ear disease in NW-Europe is Fusarium Head Blight (FHB), caused by a complex of pathogens consisting of *Fusarium* and *Microdochium* species (Bottalico and Perrone, 2002, Champeil et al., 2004). FHB causes yield losses by infecting florets during anthesis, causing either sterility or interfering with developing grains. In addition, some *Fusarium* species produce mycotoxins that, if present in high concentrations, can render the grain unusable (Champeil et al., 2004). Other ear diseases which do not actively reduce yields or turn the grain inedible can affect the market value of the grain. Examples of these include sooty moulds (mainly *Cladosporium* and *Alternaria* species) which can discolour the grain making it undesirable for milling (Rees et al., 1984).

Finally diseases such as eyespot, Fusarium foot rots and Take-All target lower stems, stem bases and roots. Common eyespot (*Oculimacula*

acuformis and O. yallundae) is perhaps the most severe of the lower stem-based diseases, not only increasing lodging risk by softening the stem base tissue but also restricting nutrient and water uptake to the canopy (Paveley et al., 2012). The most important disease affecting the root system is take-all (*Gaeumannomyces graminis* sp. *tritici*) that affects water and nutrient uptake in the roots, which in turn cause ear bleaching during grain filling (Cook, 2003).

2.2.3 Septoria tritici blotch (*Zymoseptoria tritici)*

Zymoseptoria tritici, recently renamed from *Mycosphaerella graminicola,* (anamorph *Septoria tritici*) is a fungal pathogen belonging to the Dothideomycetes, the largest class of ascomycete fungi. *Zymoseptoria* tritici belongs to the genus *Mycosphaerella* which comprises of over 10,000 members, many of which are plant pathogens infecting a diverse range of plant species of economic importance, such as banana (*M. fijiensis, M. eumusae),* citrus species (*M. citri, M.horii)*, coffee (*M. coffeicola)*, cotton (*M. gossypina)* and strawberry (*M. fragarie*). The genus *Mycosphaerella* was recently shown to be polyphyletic, even containing different groups of Dothidoemycetes, and subdivided into groups largely based on their anamorphs (Crous et al., 2007). Following the subdivision of *Mycosphaerella,* the genus *Zymoseptoria* was proposed, including *Septoria*-like species infecting graminicolous hosts (Quaedvlieg et al., 2011).

Zymoseptoria tritici is the causal agent of septoria tritici blotch (STB) in wheat, currently the most important wheat disease in Western Europe. Estimates indicate that 30-50% of wheat grain yield could be lost to this pathogen if left untreated (Eyal et al., 1987, Burke and Dunne, 2008). It has been estimated that there is an annual cost of €120 - €240M of harvest losses due to this pathogen in the UK alone (Fones and Gurr, 2015). Septoria tritici blotch is characterised by necrotic lesions surrounded by chlorotic tissue, and a typical sign of STB are pycnidia and pseudothecia embedded in the necrotic lesions (Fig 2.2). Infection of emerging leaves occurs throughout the season, but leaves do not display disease symptoms for about two weeks after this due the non-symptomatic latency period of STB (Ponomarenko et al., 2011), and the disease symptoms remain in the lower canopy. Septoria tritici blotch primarily impact the yields by reducing photosynthetic area of the leaves that contribute to grain filling (Paveley et al., 2001). *Zymoseptoria tritici* has a hemibiotrophic lifestyle evading plant defences for approximately two weeks

following infection in a biotrophic manner, after which it switches into a necrotrophic phase and causes chlorosis and necrosis of the plant tissue (Orton et al., 2011).



Fig. 2.2. Visible symptoms caused by *Zymoseptoria tritici.* Photograph of Septoria tritici blotch symptoms in the lower wheat canopy at ear emergence.

The genome of *Z. tritici* is 39.7 Mb, and contains 21 chromosomes. The full genome of *Z. tritici* was published in 2011 and showed a remarkable plasticity in the genomic material (Goodwin et al., 2011). Out of the 21 chromosomes, there are 13 core and 8 dispensable chromosomes. The dispensable chromosomes can seemingly be lost without any discernible effect on the fungi (Goodwin et al., 2011). It is not known what functions they confer, but it is speculated that the dispensable chromosomes allow for a "two-speed evolution"; maintaining a genetic stability in the core chromosomes and a high mutation rate in the dispensable chromosomes (Testa et al., 2015). There are higher proportions of repeated sequences and transposable elements in these chromosomes compared to the core chromosomes (Dhillon et al., 2014). No *Z. tritici* pathogenesis genes have been annotated on the dispensable chromosomes, which is surprising as they are often found there in other pathogens, for instance *F. oxysporum* (Testa et al., 2015). The *Z. tritici* pathogenesis genes seem to be primarily located in the core chromosomes; Mirzadi Gohari et al. (2015) recently identified 78 genes of interest and 8 QTLs all of which were located in the core chromosomes.

2.2.3.1 Lifecycle of Zymoseptoria tritici.

The infection process starts with a fungal spore (either a sexual ascospore or an asexual pycnidiospore) landing on a wheat leaf. Under suitable conditions, spores germinate within 24 hours and form hyphae which penetrate the host leaf through stomatal openings (Kema et al., 1996a). Appressorium-like structures can be formed by the fungi, but do not seem to be necessary for successful penetration (Kema et al., 1996a). The hyphae continue to spread intercellularly throughout the leaf for between 10-14 days before visible symptoms appear (Orton et al., 2011). There is no penetration of mesophyll or epidermal cells during this biotrophic phase and the pathogen is likely to obtain nutrients from the apoplastic space (Ponomarenko et al., 2011). Transcriptomic studies show an up-regulation of peptidases, lipases, and amylases, indicating that *Z. tritici* feeds on host proteins, lipids, and sugars (Yang et al., 2013).

The pathogen then switches to the necrotrophic phase causing leaf cells to collapse and release nutrients. It is currently unknown what triggers the switch from a biotrophic to a necrotrophic stage (Ponomarenko et al., 2011). It has been suggested that *Z. tritici* releases toxins as cells in the proximity of the pathogen, but not actually in contact with, also collapse during this stage (Kema et al., 1996a). There have been reports of *Z. tritici* releasing cell wall-degrading enzymes, such as xylanase and pectinase, during *in vitro* studies (Douaiher et al., 2007). Brunner et al. (2013) showed that there is a distinct difference in which cell wall-degrading enzymes were expressed during the biotrophic and necrotrophic pathogen stages, indicating that these enzymes have life cycle-specific uses. Genome sequencing, however, showed a distinct decrease in genes encoding cell wall-degrading enzymes in *Z. tritici* compared to other fungal pathogens (Goodwin et al., 2011). After this switch to necrotrophy, the hyphal mass continues to proliferate within the leaf and 14-28 days post infection conidia are produced in substomatal cavities of senescent tissue, which then release pycnidiospores. These asexual pycnidiospores are the primary means of re-infection during the latter stages of the crop growth and from yield formation as pseudothecia (that release the sexual, airborne ascospores) take considerably longer to develop (Ponomarenko et al., 2011).

2.2.3.2 Epidemics

Wheat is first infected by *Z. tritici* by airborne ascospores released from remaining wheat debris and volunteer plants in the autumn (Hunter et al., 1999). These ascospores can travel over long distances (Ponomarenko et al., 2011) and are therefore their incidence is difficult to prevent. There may also be available pycnidiospores from infected wheat stubble remaining in the same field. After the initial infection from external sources, the pathogen moves up through the canopy by re-infection of pycnidiospores and ascospores throughout the season (Fig 2.3). Pycnidiospores are transferred by rain splashes and therefore limited to re-infection of the same plant, or neighbouring ones. Studies indicate that pycnidiospores will not normally spread further than 1 m^2 from the host plant (McDonald and Linde, 2002a). Pycnidiospores may be transferred to neighbouring plants in dry weather conditions via leaf contact, so called horizontal transport. Some weeks later, new ascospores are released from psuedothecia on the infected plant (Hunter et al., 1999). As these are airborne, they will spread the disease over a considerably longer distance.

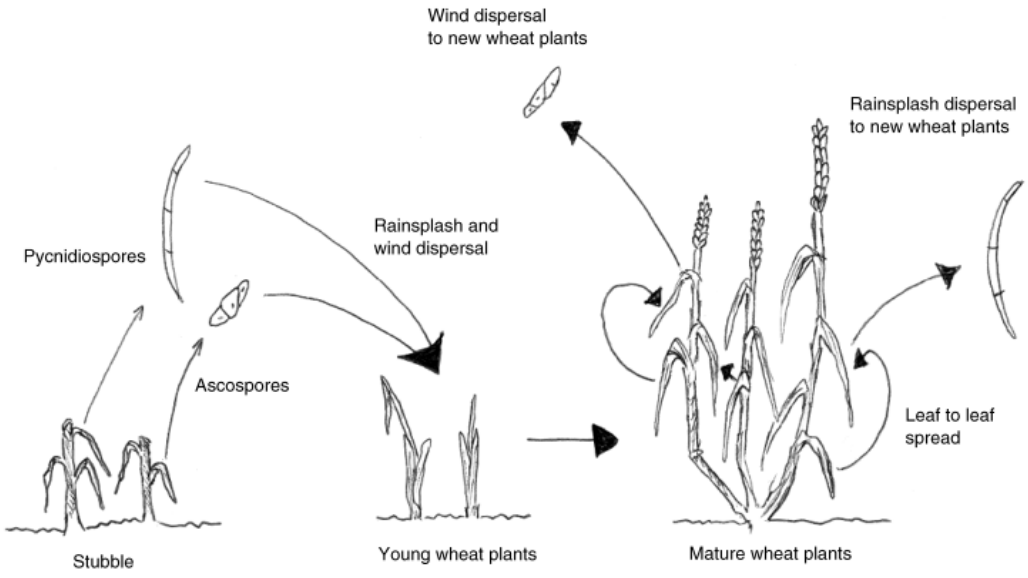


Fig 2.3. The life cycle of *Zymoseptoria tritici,* from Palmer and Skinner (2002).

2.2.3.3 Fungicide resistance

Currently control of STB is mainly managed by fungicides as few commercially available winter wheat varieties have sufficient resistance against STB. The most resistant variety on the UK recommended list (2014-

2015) is Cougar with a rating of 7 (on a scale of 1-9, www.hgca.com) and similarly in Ireland, Sy Epson is the most resistant recommended variety, also with resistance rating 7 (Recommended list 2014-2015, www.agriculture.gov.ie). Due to the current fungicide dependency, it is important that fungicides provide efficient protection against *Z. tritici.* However, fungicide resistance is becoming increasingly common and will become a major problem if not properly managed.

The quinones outside inhibitor (QoIs) are an example of a group of fungicides whose mode of action has already been overcome by *Z. tritici*. The QoIs target the Qo site in the mitochondrial cytochrome bc1 complex, inhibiting the electron transport and consequently mitochondrial respiration (Fernández-Ortuño et al., 2010). Resistance to the QoIs is conferred by a single point mutation at cytochrome *b* G143, causing an amino acid change of glycine to alanine. *Zymoseptoria tritici* populations started to develop resistance to these fungicides within 4 years of use on wheat crops in NW-Europe (Lucas, 2006). In Europe, QoIs are today considered to be inefficient against *Z. tritici* and are no longer used widely, while the first resistant *Z. tritici* populations in North America and Northern Africa were reported in 2013 (Taher et al., 2014, Estep et al., 2015).

The most common fungicides used for STB disease control are the azoles. These have been used successfully for the last 30 years, and unlike the QoIs, *Z. tritici* resistance has developed gradually (Stammler and Semar, 2011). All azoles inhibit fungal growth within the leaf by targeting a key enzyme (sterol 14α-demethylase, also known as CYP51) in sterol biosynthesis, needed for cell membrane formation (Clark et al., 2010). A decrease in the efficiency of many azoles was observed in the late 1990's. Today, most azoles, including epoxiconazole and prothioconazole which have been fully effective until recently, show signs of decreased efficiency (www.frac.info). Unlike the QoIs it has taken combinations of several different mutations in CYP51 to confer resistance to azoles, which affect the efficacy of the azoles differently (Cools and Fraaije, 2013).

With the continued erosion of azole efficacy, increased reliance for STB disease control is placed upon the succinate dehydrogenase inhibitors (SDHIs). The SDHIs target fungal respiration by blocking mitochondrial complex II in the mitochondrial electron transport chain. Although some older generation SDHIs (such as carboxin) have been around since the 1960's, no

Z. tritici field resistance has been observed (Fraaije et al., 2012) but resistance in laboratory isolates have been found (Skinner et al., 1998, Fraaije et al., 2012, Scalliet et al., 2012). Since 2012, *Z. tritici* strains with slightly reduced sensitivity to SDHIs have been found in UK, France, Ireland and Germany (www.frac.info). In 2015, the first SDHI insensitive *Z. tritici* isolates were identified in Ireland (Dooley et al., 2016b).

The risk of SDHI resistance developing in *Z. tritici* was highlighted by Fraaije et al. (2012) who generated mutations by UV radiation, and found 16 mutations conferring resistance to carboxin. Similarly, Scalliet et al. (2012) studied resistance of new generation SDHIs (as well as carboxin) by UV mutagenesis and found 27 mutations conferring resistance. FRAC (Fungicide Resistance Action Committee) has declared SDHIs to be of medium to high risk of developing resistance (www.frac.info).

2.2.3.4 Molecular studies and detection of Zymoseptoria tritici

Early studies of *Z. tritici* used restriction fragment length polymorphism markers to determine the genetic variation in field populations (McDonald and Martinez, 1990b). For instance, McDonald and Martinez (1990a) found 22 haplotypes in 93 isolates collected from a single field. The high genetic variability of *Z. tritici* populations would later be confirmed by numerous publications worldwide (Chen and McDonald, 1996, Schnieder et al., 2001, Zhan et al., 2003). Conventional PCRs gained rapid popularity in early to mid-1990's and started to become widespread in plant disease studies (Henson and French, 1993). For *Z. tritici,* PCR based methods have been used extensively for population genetics studies. Examples include studies of sexual reproduction in *Z. tritici* ascospores, geographical distribution of mating types and pathogen evolution studies (Kema et al., 1996b, Waalwijk et al., 2002, Zhan et al., 2002, Stukenbrock et al., 2007).

Fungicide resistance is, as previously discussed, a major issue in *Z. tritici* – wheat cropping systems, and this can be seen in the numerous publications studying isolate sensitivity to the various fungicides (Rohel et al., 2002, Fraaije et al., 2005, Kildea et al., 2010, Cools et al., 2011, Fraaije et al., 2012, Dooley et al., 2015, Dooley et al., 2016a). The distribution of isolates carrying mutations conferring fungicide insensitivity has been studied using PCR-based methods, and in particular, quantitative PCR has proven useful. For instance, Fraaije et al. (2005) used quantitative PCR to determine the frequency of a mutation conferring fungicide resistance to QoI fungicides.

Disease detection using molecular methods is considered a useful alternative to visual disease assessments, as molecular methods generally have the capacity to detect pathogen presence previous to symptom development. One of the earlier *Z. tritici* detection studies using a combination of PCR and Picogreen assays was performed by Fraaije et al. (1999) and conventional PCR have also been used to detect differences in *Z. tritici* infection in various UK wheat varieties and effectiveness of fungicide treatment on infection (Fraaije et al., 2001, Rohel et al., 2002). Real-Time PCR has been particularly encouraging as a detection method, and was used by Bearchell et al. (2005) to determine the amount of *Z. tritici* to *Stagonospora nodorum* in historical samples. The following year, Guo et al. (2006) detected *Z. tritici* DNA pre-symptomatically, and showed a reasonable (although non-linear relationship) between pathogen DNA and visible disease symptoms. Abd-Elsalam et al. (2011) developed a qPCR assay for in-leaf detection of *Z. tritici* which showed high species specificity and also a low limit of detection. The detection of *Z. tritici* does not however need to be in-leaf; detecting airborne inoculum can be very useful in pathogen monitoring. For instance, Duvivier et al. (2013) used spore traps to quantify distribution of airborne inoculum by the use of qPCR.

2.2.4 Disease management

To ensure the profitability of wheat production, growers in NW Europe are dependent on fungicides for disease control. To maximise the genetic progress made in wheat yield potential, more inputs are needed and crops have become increasingly susceptible to disease over time. In general, high yielding varieties tend to be more susceptible to disease than low yielding varieties (Gaunt, 1995). Over-dependence on fungicides for disease control comes with the risk that the target pathogens may develop resistance to fungicides, which could have a devastating effect on crop yields. Many important cereal pathogens are already resistant to a number of major fungicides (Ma and Michailides, 2005). Due to the site-specific actions of most modern fungicides they are quite easy to overcome, it is now generally recommended to use a mixed spray schedule using combinations of fungicides with different modes of actions in order to reduce selection for insensitive pathogen strains (van den Bosch et al., 2014).

Winter wheat growers in the UK and Ireland are generally recommended to apply three to four fungicide sprays per season. These

sprays are referred to as T_1-T_3 (or T_0-T_3 for a four-spray schedule), aimed to protect the upper three leaves of the canopy as they are the major contributors to the final yield (Shaw and Royle, 1987, Cook et al., 1999). The first spray in a four-spray schedule is T_0, applied approximately at GS30. This spray is often combined with plant growth regulators, and is primarily a preventative measure for foliar diseases, in particular for STB. The following fungicide application (or first spray in a three-spray schedule) T_1, is applied after the final leaf 3 is fully emerged (around GS32) and is mainly aimed at foliar diseases such as STB and rusts, but also protection against stem diseases such as eyespot (Paveley et al., 2012). The subsequent spray, T_2, is then applied at GS39, when the flag leaf is fully emerged. This spray is aimed at protecting the final two leaves from foliar diseases, and is considered the most important spray from the aspect of protecting potential yields. The last spray (T_3) is applied after ear emergence, optimally mid-flowering, GS61-65. This spray has a dual function; additional protection for the top two leaves (STB and rust), and as protection against ear diseases, in particular Fusarium head blight (Paveley et al., 2012).

Diseases can to some extent be managed by agronomic practises. For instance, crop rotation by growing different crops each season where the carry-over and re-infection of pathogens from the previous field season is minimised. This is most effective for soil-based diseases such as take-all where fungicides are only partly effective (Cook, 2003). Another farming practice known to reduce disease levels of pathogens such as take-all and Septoria tritici blotch is delayed sowing date (Shaw and Royle, 1993, Cook, 2003). A late sowing date might, however, affect crop establishment and yields negatively and might therefore not be economically viable (Spink et al., 2000). Similarly, there is some evidence that high rates of nitrogen fertilisers benefit pathogen development (Walters and Bingham, 2007), but due to the high nitrogen requirement of modern high-yielding cultivars it is not feasible to apply lower than recommended nitrogen doses.

2.3 Breeding for decreased fungicide dependence

2.3.1 Disease resistance

Exploiting the natural self-defence systems of plants is environmentally-friendly and a sustainable approach to crop protection. The most well-known, and well-studied, self-defence is disease resistance in which the host actively resists pathogen attacks and thereby limits infection. There are two types of disease resistance; qualitative, isolate-specific resistance and quantitative, non-specific resistance.

Qualitative resistance actively deters pathogens from successfully infecting the plant on a molecular, gene-for-gene basis. Qualitative resistance (R) genes encode R-proteins which recognise specific avirulence (avr) proteins expressed by pathogens and trigger defence responses (Jones and Dangl, 2006). Only pathogen strains expressing a specific avr-protein will be recognised by a host plant; and qualitative resistance is therefore considered to be isolate-specific (Gururani et al., 2012). Isolate-specific resistance genes have been introduced to crops by breeders for some time, as they are easy to select for with clear, resistant phenotypes, but are generally overcome by pathogens quite rapidly (Boyd et al., 2013). Pathogens that have a mixed reproduction lifestyle (i.e. both asexual and sexual reproduction) and/or are highly mobile (e.g. airborne spores) are likely to be able to rapidly overcome isolate-specific resistance genes (McDonald and Linde, 2002b). Pathogens with long distance dispersal of asexual spores, such as *P. striiformis* are most damaging. Growing large areas of crops with single R-genes increases the selection of virulent pathogen strains, through directional selection of the pathogen. Furthermore, increasing use of varieties with the same R-gene may lead to so-called “boom and bust” cycles (McDonald and Linde, 2002a). These occur when a certain resistance gene becomes widespread and the selection pressure on the pathogen population therefore increases. Once virulence in the pathogen population finally occurs, it spreads rapidly until the resistance gene is broken (McDonald and Linde, 2002a). A common example of a boom and bust cycle is the *Yr17* resistance gene in wheat, which conferred resistance to yellow rust in the UK. *Yr17* became widely used in late 1980’s to mid-1990’s, and by late-1990 was grown on 35% of the total acreage in the UK (Bayles et al., 2000). When virulence emerged in the rust population, the cultivars relying on the *Yr17* gene rapidly became highly susceptible. In particular, the widely grown UK cultivar Brigadier’s yellow rust resistance

rating dropped to 1 on the UK Recommended List scale of 1-9 within three years of its release (Bayles et al., 2000).

In order to deploy qualitative resistance without the high risk of breakdown, it has been suggested that pyramiding (or stacking) a number of resistance genes would limit the emergence of virulence in pathogen populations (Liu et al., 2000). While this has been successful in certain pathosystems, it might not be durable for pathogens such as *Z. tritici* which have already overcome most known major resistance genes (Brown et al., 2015). Gene rotations have been suggested in which cultivars of different genetic backgrounds would be grown in rotation in order to reduce directional selection (McDonald and Linde, 2002a), and host mixtures with differential pathogen resistance could also reduce disease pressure (Mikaberidze et al., 2015).

Quantitative (QR, or partial) resistance genes confer incomplete resistance and can range from very small to major effects. This type of resistance is often considered to be more durable due to the smaller effects of genes, leading to reduced pathogen selection pressure. Quantitative resistance is also considered to be broad-spectrum, i.e. non- isolate specific, thereby further reducing selection pressure (Poland et al., 2009). It is likely that quantitative resistance is conferred in a range of different ways, unlike R-genes that tend to act on a traditional zigzag basis. The general consensus is that quantitative resistance is conferred by general defence mechanisms that could be structural and/or conferred by chemicals or proteins, resulting in reduced infection and/or delayed pathogen development (Poland et al., 2009).

A common defence mechanism conferred by quantitative resistance is increased reinforcement of the cell wall, in order to stop penetration by pathogens. Strengthening of host cell walls is a part of the basal (or “non-host”) resistance response, the first line of defence triggered by pathogen recognition (Heath, 2000). One such QR response is conferred at the *Mlo* gene locus in barley. The loss of function *mlo* allele confers highly-effective, broad-spectrum resistance to powdery mildew and has been proven durable (Collins et al., 2007). Similar resistance to leaf rust is conferred by the wheat genes *Lr34* and *Lr46*, which although less efficient, have been proven durable and multi-pathogen resistant, as they also reduce powdery mildew infections (Collins et al., 2007). Quantitative resistance has also been suggested to be conferred through chemical means, by host secretion of toxic and/or detoxing

compounds in order to reduce pathogen invasion. On the other hand, it has been suggested that quantitative resistance could simply be conferred by weak (or “leaky”) R-genes (Poland et al., 2009).

One of the reasons why qualitative resistance has been more targeted by breeders than quantitative resistance is that while R-genes display distinct phenotypes, quantitative resistance generally displays a wide range of phenotypes (Singh, 2012). Another is the need to combine several quantitative resistance genes in order to achieve a satisfactory level of resistance (Singh, 2012). Furthermore, quantitative resistance is often variable, with a high degree of genotype x environment interaction, and expression might therefore vary between sites and seasons (Cherif et al., 2010).

2.3.2 Disease escape

Disease escape is a passive defence based on the actual avoidance of pathogen spores. Plants can escape pathogen spores by various means, at times causing susceptible plants to appear resistant (Agrios, 1988). Sometimes the non-presence of the pathogen is referred to as escape, for instance sowing out of synchrony with the pathogen lifecycle (Parry, 1990). Other means of disease escape can be morphological, for example in closed flowering barley varieties that avoid loose smut infection by limiting access to the flower (Parry, 1990).

In the wheat - *Z. tritici* pathosystem disease escape generally refers to the limiting of upward transfer of inoculum through the canopy. Tall crops tend to incur less disease in the upper canopy as fewer spores are able to reach the top leaves, and are therefore said to employ disease escape (Arraiano et al., 2009). Similarly, if a wheat cultivar displays canopy traits that limit spore transfer within and between leaves, such as shorter and/or less erect leaves, it might be considered disease escape (Lovell et al., 1997). Even though the concept of disease escape is well established, the actual term is perhaps less so. A recent paper attributed 18% of observed variation in resistance to Septoria tritici blotch to increased plant height, using the term “morphological resistance” (Miedaner et al., 2013). The authors did, however, attribute the lack of infection to the limitation of upward transfer of pycnidiospores, thereby essentially describing a disease-escape mechanism

2.3.3 Disease tolerance

Disease tolerance is determined by how well a susceptible crop tolerates a specific disease. It has been defined as “the ability to maintain yield in the presence of disease symptoms” (Parker et al., 2004). The concept of disease tolerance was proposed to explain observations of varietal differences in the relationship between disease severity and yield (Bingham et al., 2009). As early as 1894, Cobb suggested that the term rust-enduring could be applied to certain Australian wheat varieties which were able to maintain good yields despite being susceptible to rust infections (reviewed in Schafer (1971)). Disease tolerance has since been observed in crops in respect of a diverse range of pests, from fungi (rusts, STB, *Septoria nodurum,* powdery mildew) to viruses, nematodes and herbivores (Schafer, 1971, Bingham et al., 2009).

Despite the many observations of disease tolerance, there is some confusion about the actual definition of the term. ‘Disease tolerance’ tends to be used differently in different fields of plant science. For instance, many literature sources tend to use disease tolerance and partial resistance interchangeably (Mussel, 1980). The term is particularly used in molecular plant pathology as passive defence at the cellular level, for instance, by structural defences and tolerance to pathogen toxins (Greenberg and Yao, 2004).

2.3.3.1 How is tolerance quantified?

Tolerance is expressed as the slope between disease severity and a plant fitness trait, often yield (Inglese and Paul, 2006). Disease severity is often calculated from the Area under Disease Progress Curve (AUDPC), which estimates disease development as a percentage of leaf area integrated over time. In order to relate disease severity to yield, the actual canopy green area could be calculated from the Healthy Area Duration (HAD) curve, which estimates loss of healthy tissue over time. The HAD curve relates more directly to the photosynthetically active radiation (PAR) than AUDPC, and should therefore be a better estimation of pathogen induced damage on yield by loss of green area (Waggoner and Berger, 1987).

As can be seen from the methods above, disease tolerance quantification relies heavily on visual assessments, either of disease severity or green canopy area loss. As these are based on visual estimations they are

prone to subjective errors, particularly when assessing cultivars of different leaf sizes (Cooke, 2006). Another weakness of “area over time” curves is the inability to distinguish a high green leaf area index (GLAI) that is lost quickly from a low GLAI that is lost slowly (Bryson et al., 1997). Furthermore, visual disease scores might not accurately reflect the actual fungal biomass within the host leaves, especially so for pathogens with long latency periods (Cooke, 2006, Oliver et al., 2008). In order to accurately quantify disease severity, other methods could be used; for example. molecular methods such as quantitative PCR (Bearchell et al., 2005, Guo et al., 2006, Oliver et al., 2008), or histological methods such as microscopy using fluorescent markers (Ayliffe et al., 2013)

2.3.3.2 Which crop traits confer tolerance?

Despite numerous observations of disease tolerance, there is still relatively little information published on which mechanisms and traits confer tolerance. It is generally agreed that physiological traits at the plant organ, single plant and crop scale underlie disease tolerance, unlike resistance with is conferred at the molecular and cellular scale. A number of physiological traits such as increased green canopy area, compensatory up-regulation of leaf photosynthesis in residual green area, and increased utilization stem carbohydrate reserves have been suggested, but no findings have been conclusive (Bingham et al., 2009). Furthermore, it is likely that traits conferring disease tolerance will differ between cereal species (Bingham et al., 2009). For instance, the most important stages of yield formation in wheat are during late stem-elongation when growth affects floret survival and grains m^{-2} and during the grain-filling period. Therefore, pathogens causing loss of green area during this time have a large impact on the final yield, especially in the top leaves of the canopy (Shaw and Royle, 1989). For this reason, it is likely that potential tolerance traits act around this time. On the other hand, the yield potential of spring barley is determined earlier in the season, with growth during early stem elongation influencing tiller survival and grains m^{-2}, and tolerance traits in barley would therefore primarily act at that time (Bingham et al., 2009, Bingham and Topp, 2009).

Regardless of which life stage a pathogen attack occurs in, the loss of green photosynthetic leaf area is likely to be a major factor in yield penalties. A potential tolerance mechanism is the ability to cope with loss of green area by up-regulation of photosynthesis in non-infected tissues (Bingham et al.,

2009). There is some evidence for this in barley for powdery mildew infections, where the photosynthesis rate in a non-inoculated third leaf in an infected seedling was higher than the third leaf of healthy plants (Williams and Ayres, 1981). In wheat seedlings, it was shown that a healthy fourth leaf on a plant inoculated with *Septoria nodorum* had a higher photosynthetic rate than the corresponding leaf on a healthy plant. These authors did not, however, find the same response on inoculated plants at the six-leaf stage or later (Williams and Ayres, 1981). Scholes and Farrar (1986) found that while the total net photosynthesis of a barley leaf decreased when infected by brown rust, the photosynthesis rate per unit chlorophyll was increased in the same infected leaf. It should be noted, however, that the above-mentioned studies did not quantify tolerance and were performed on single cultivars.

A later study using three different oat lines (two cultivated oats and one wild oat) showed that a more tolerant oat line was able to maintain a higher net photosynthesis rate and had less chlorophyll loss while infected by powdery mildew, than another less tolerant oat cultivar. The wild oat, despite having the highest disease severity, was shown to be the most tolerant and was also able to maintain photosynthesis and chlorophyll levels for a longer period of time than the tolerant cultivated oat line (Sabri et al., 1997). Similarly, a study on two wild and one modern barley variety, in which the wild lines displayed higher and lower disease tolerance than the modern barley variety, attributed disease tolerance in these genotypes to the ability to slow chlorophyll loss and early senescence caused by disease (Akhkha et al., 2003). On a different note, Inglese and Paul (2006) studied the effect of two different rust fungi (one native and one alien) on a single genotype of *Senecio vulgaris* and found differences in the plant response to the two rust species. Despite the slightly higher infection rate of the native fungus, the plant lost photosynthetic capacity and had decreased growth when introduced to the alien fungus. In addition, there was evidence of up-regulation of photosynthesis in non-infected leaves when infected with the alien but not with the native fungus (Inglese and Paul, 2006). This could indicate that there are differences in disease tolerance response to different pathogens in a single genotype, which has not been extensively studied, and is in contrast to the general opinion that disease tolerance could act as a broad-spectrum defence mechanism (Bingham et al., 2009).

Zuckerman et al. (1997) found contrasts in disease tolerance between two Israeli spring wheat cultivars, Miriam and Barkai, in a controlled environment experiment. The tolerant genotype Miriam was able to maintain an equal grain weight and harvest index when infected by Septoria tritici blotch. The authors attributed this apparent disease tolerance to the ability of this genotype to maintain a high carbon fixation rate (three to five-fold higher than the intolerant genotype in the same study) in the residual green leaf area. However, when Foulkes et al. (2006) studied radiation-use efficiency in field experiments of winter wheat in the UK they found the opposite relationship – a low RUE in the absence of disease was associated with high disease tolerance.

In addition to enhanced photosynthesis, it has been suggested that a larger leaf area might be a tolerance-conferring trait. At least in theory, under the same amount of disease, a cultivar that has a larger green canopy area will have a larger actual green photosynthetic area left and would be able to maintain a higher radiation interception and canopy photosynthesis (Foulkes et al., 2006, Bingham et al., 2009). Larger flag leaf area has been positively correlated with increased tolerance of Septoria tritici, but seems to display a negative relationship with yield (Foulkes et al., 2006).

Recently, there have been suggestions that the grain source to sink balance could be a factor in determining disease tolerance (Paveley et al., 2008). Modern wheat varieties have been shown to be closely balanced between source and sink in the light-limited conditions of NW Europe (Beed et al., 2007). Paveley et al. (2008) suggested that a high source to sink balance in the form of healthy area duration (HAD) per grain could be conducive to disease tolerance since genotypes with additional source capacity would be able to maintain grain-filling despite green area loss. A tightly balanced genotype would however become source limited more quickly and have reduced grain-filling capacity.

Another way of buffering the effect of pathogen infections could be increased accumulation and/or utilization of stem storage of water-soluble carbohydrates. A greater supply of redistributable assimilates in the stem might buffer the loss of assimilate from lost photosynthetic tissues (Foulkes et al., 2006, Bingham et al., 2009)**,** but this has not been shown in tolerance studies. For example, a study of a range of wheat genotypes indicated a negative relationship between stem WSC and tolerance of Septoria tritici in

the UK (Foulkes et al., 2006), while there was no relationship at all in Israeli spring wheat (Zuckerman et al., 1997).

2.3.3.3 Tolerance in existing wheat varieties

Parker et al. (2004) presented differences in tolerance in UK winter wheat cultivars by screening a number of varieties for tolerance against *M. graminicola*. None of those varieties are, however, currently on the HGCA recommended list (www.hgca.com). There is a lack of recent literature describing the level of tolerance in current germplasm, and few reported studies incorporate more than a few genotypes in each experiment. The largest peer-reviewed study of disease tolerance in winter wheat to date was 16 cultivars of winter wheat based in the UK (Parker et al., 2004). One of the early studies performed by Ziv and Eyal (1976) showed differences in disease tolerance for 5 Israeli spring wheat varieties. A majority of disease-tolerance studies have been performed using mapping populations and crosses between tolerant/intolerant genotypes (Ziv et al., 1981, Foulkes et al., 2006, Paveley et al., 2008).

Tolerance has not been selected for in plant breeding programmes in NW Europe, but there is a general consensus that wheat developed in (or for) developing countries has the potential to be more tolerant, as they are selected for in the absence of fungicides. Small-scale farmers in developing countries have limited access to, or funds for use of fungicides or high amounts of inputs (Huang et al., 2002), even though agrochemical use in developing countries has increased over the last decades (www.fao.org). About 75% of all wheat used in developing countries stems from CIMMYT material (www.cgiar.org). CIMMYT selects for yield in the absence of fungicides in alternate cycles, (i.e. in the presence of disease every other year), and is therefore in essence co-selecting for tolerance.

It is possible that traits from CIMMYT wheats can be identified, which break the apparent linkage between yield potential and disease tolerance, and that physiological traits that improve grain number (sink) associated with a positive effect on photosynthetic capacity (source) will maintain or increase disease tolerance. However, the adaptive wheat traits for developing countries (i.e. phenology, plant height) are different than those for NW Europe, and novel tolerance traits would need to be introgressed into adaptive winter wheat backgrounds before testing in Europe.

2.3.3.4 Utilising disease tolerance

Disease tolerance has potential to be a durable method of minimising disease impact, especially as certain tolerance mechanisms have been shown to be effective against a range of pathogen species. Therefore, any changes in the spectrum of important pathogens that are likely to occur over time would not result in loss of effect. Furthermore, it has been suggested that crop protection conferred by disease tolerance would exert little or no selection pressure on pathogen populations (Bingham et al., 2009). There is no evidence that disease tolerance and disease resistance need to be mutually exclusive, in fact Bingham et al. (2009) caution against breeding for tolerance while disregarding disease resistance. If disease tolerance and (in particular) quantitative resistance were to be combined, disease tolerance would be an excellent method to cope with remaining disease symptoms (Ney et al., 2013).

One of the drawbacks of tolerance at present is the apparent relationship with low yield potential (Parker et al., 2004, Bingham et al., 2009). Many of the putative tolerance traits have been selected against by breeders, as modern varieties have tightly balanced source-sink relationships and morphological traits selected for high grain sink size and high yields. It is therefore important to identify tolerance traits that are not linked to low yield potential (Bingham et al., 2009). Another caution against selection for disease tolerance is the potential of increased disease pressure on intolerant cultivars (Bingham et al., 2009), which could increase the rate of major resistance gene break down (McDonald and Linde, 2002b).

2.3.3.5 Tolerance and herbivory

Unlike fungal and bacterial disease pathosystems, tolerance is a relatively well known mechanism for plants to cope with herbivore damage (Fornoni, 2011). The term tolerance can encompass herbivory, for instance “the ability to tolerate a certain extent of damage without a corresponding impact on plant fitness”. However, tolerance of herbivores tends to define plant fitness as ecological fitness or biomass, in contrast to crop scientists who primarily refer to grain yield (Ney et al., 2013). As for crop disease tolerance, herbivore tolerance (in particular insect damage) has been suggested to be related to physiological traits such as resource allocation, photosynthetic activity and plant architecture (Fornoni, 2011).

More advances have been made on the genetic basis of herbivory tolerance compared to disease tolerance, and studies show that tolerance has

a heritable, genetic background (Fornoni, 2011). Several single genes have been shown to influence resource allocation in response to herbivory; for instance, Schwachtje et al. (2006) showed that the kinase SnRK1 was directly involved in up-regulated root allocation in tobacco in response to herbivore damage. Recently, Siddappaji et al. (2013) suggested that the overcompensation mechanism in *Arabidopsis thaliana* was due to an up regulation of plant metabolism in response to insect damage regulated by the glucose-6-phopsphate dehydrogenase. Regardless of whether plant damage was incurred from herbivores or disease, in most cases it will cause loss of photosynthetic tissue and at least part of the underlying tolerance mechanisms are likely to be the same or at least similar (Ney et al., 2013).

3. Materials and Methods

3.1 Overview of experiments

Field experiments were conducted in 2011/2012, 2012/2013, and 2013/2014. The objective of the first two experiments was to screen a number of doubled-haploid lines and cultivars of wheat for disease tolerance to Septoria tritici blotch (STB) and physiological traits. In the final year, lines displaying high and low tolerances were selected for further in-depth phenotyping of an extended range of physiological traits to identify traits conferring disease tolerance to STB.

Three glasshouse experiments were conducted in 2014. The first two experiments studied the relationship between disease symptoms and inoculum density in the winter wheat cultivars Rialto and Xi19. The third experiment investigated differences in disease symptoms and rate of disease development in eight winter wheat cultivars and two doubled-haploid lines of diverse genetic background. In these experiments, a Real-Time quantitative PCR (qPCR) assay was developed in order to quantify levels of *Zymoseptoria tritici* (STB) in wheat leaf samples. In order to determine the accuracy of the assay, leaves of the plants studied in the glasshouse experiments were used to determine the relationship between *Z. tritici* DNA and visible disease symptoms. Finally, the STB pathogen load in flag leaf samples collected from field trials in 2012 and 2014 were quantified using the developed qPCR assay.

3.2 Field experiments

3.2.1 Sites and crop management

In 2011/2012, the field experiment was located at Teagasc Oak Park, Carlow, Ireland (58° 37' 40" N, 7° 0' 45" W). The field experiment was drilled on 1st November 2011 following a crop of winter oilseed rape, and harvested on 30th August 2012 (48 out of 300 sub-plots) and 4th September 2012 (remaining sub-plots). The sub-plots were 2.2 x 9 m and sown at a seed rate of 330 seeds m^{-2}. The clay loam soil had P and K indexes of 3, and 25 kg ha^{-1} of P and 108 kg ha^{-1} of K was applied to the sub-plots. The soil had a nitrogen index of 2, and a total of 165 kg N ha^{-1} was applied (Sulfa CaN 26.6% N, 5%

S), over three splits. Two splits, 66 kg N ha^{-1} each, were applied on 20th March 2012 and 16th April 2012, and the final split (33 kg N ha^{-1}) on 16th May 2012.

The 2012/2013 field experiment was also located at Teagasc Oak Park, see details above. The field trial was drilled the 15th November 2012, following winter oilseed rape. The sub-plots were 2.2 x 9 m, and sown at a seed rate of 330 seeds m^{-2}. The clay loam soil had a P index of 4; hence no additional P was added. The K index was 2, and a total of 125 kg ha^{-1} of K was applied. The soil had a nitrogen index of 2, and 200 kg ha^{-1} of N (Super Net 27% N + 3.7% S) was applied to the experiment over three splits. The first two splits (60 kg N ha^{-1} each) were applied on 15th March 2013 and 18th April 2013, and the final split (80 kg N ha^{-1}) was added on 10th May 2013. The trial was harvested on 28th August 2013 by a Sampo combine harvester (Sampo-Rosenlew, Finland).

The field experiment in 2013/2014 was conducted near Leominster, Herefordshire, UK (52° 16' 14" N, 2°, 51' 37" W), and was managed by ADAS Rosemaund. The experiment was sown on 10th October 2013, following a crop of winter oats. The sub-plots were 10 x 2.2 m, sown at a seed rate of approx. 460 seeds m^{-2}, and harvested on 4th September 2014 by a Sampo 2010 combine harvester (Sampo-Rosenlew, Finland). The silty clay loam soil had P and K indexes of 2, and 35 kg ha^{-1} of P and 130 kg ha^{-1} of K was applied to the plots. A total of 180 kg N ha^{-1} was applied over three splits (40, 80, 60 kg N ha^{-1} each as ammonium nitrate 34.5% N), on 3rd March, 8th April and 14th May in 2014.

3.2.2 Wheat lines, fungicide treatments, and experimental design

All field experiments were divided into two fungicide treatments; one fully treated against *Z. tritici* and one control treatment that was untreated against *Z. tritici*. Both treatments were fully protected against other wheat diseases. The same fungicides and rates were used each year (Table 3.1). All field experiments were designed as split-plot experiments, with fungicide treatments randomised on main plots and genotypes randomised on sub-plots. Each fungicide x genotype treatment was replicated 3 times.

Table 3.1. Field experiment fungicide treatments.

Fung. timing	Date in 2012	Date in 2013	Date in 2014	Untreated plots	Protected plots	Active ingredients
T_0	30/03/2012	23/04/2013	28/03/2014	Talius (0.2 L/ha)	Talius (0.2 L/ha)	Proquinazid (200 g/L)
				Unix (1.0 kg/ha)	Unix (1.0 kg/ha)	Cypronidil (750 g/kg)
				Comet 200 (0.5 L/ha)	Comet 200 (0.5 L/ha)	Pyraclostrobin (200 g/L)
					Gleam (2.0 L/ha)	Epoxiconazole (37.5 g/L), metconazole (27.5 g/l)
					Bravo (1.0 L/ha)	Chlorothalonil (500 g/L)
T_1	27/04/2012	15/05/2013	04/05/2014	Talius (0.2 L/ha)	Talius (0.2 L/ha)	Proquinazid (200 g/L)
				Comet (0.5 L/ha)	Comet (0.5 L/ha)	Pyraclostrobin (200 g/L)
					Gleam (2.0 L/ha)	Epoxiconazole (37.5 g/L), metconazole (27.5 g/l)
					Bravo (1.0 L/ha)	Chlorothalonil (500 g/L)
T_2	23/05/2012	05/06/2013	29/05/2014	Comet (0.5 L/ha)	Comet (0.5 L/ha)	Pyraclostrobin (200 g/L)
					Gleam (2.0 L/ha)	Epoxiconazole (37.5 g/L), metconazole (27.5 g/l)
					Bravo (1.0 L/ha)	Chlorothalonil (500 g/L)
T_3	12/06/2012	21/06/2013	18/06/2014	Comet (0.5 L/ha)	Comet (0.5 L/ha)	Pyraclostrobin (200 g/L)
					Gleam (2.0 L/ha)	Epoxiconazole (37.5 g/L), metconazole (27.5 g/l)
					Bravo (1.0 L/ha)	Chlorothalonil (500 g/L)

Table 3.2. Field experiment herbicides, insecticides and PGR inputs.

Field season	Type	Product	Rate (ha^{-1})	Date	Target
2012	Insecticide	Sumi Alpha	165 ml	28/11/2011	Aphids
	Pesticide	Draza	5.0 kg	28/11/2011	Slugs
	Pesticide	Draza	5.0 kg	24/01/2012	Slugs
	Herbicide	Diflanil	0.25L	28/11/2011	Broad leaf weeds
	Herbicide	Croplink IPU	2.5L	28/11/2011	Meadow grass and broad leaf weeds
	Herbicide	Axial	0.25L	27/04/2012	Wild oats
	Herbicide	Adigor	1.0L	27/04/2012	Wild oats
	PGR	CCC 750	2.0L	28/03/2012	
2013	Insecticide	Sumi Alpha	165 ml	24/01/2013	Aphids
	Pesticide	Draza	5.0 kg	20/11/2013	Slugs
	Herbicide	Flight	3.5L	24/01/2013	Meadow grass and broad leaf weeds
	Herbicide	Croplink IPU	1.5L	24/01/2013	Meadow grass and broad leaf weeds
	Herbicide	Cameo max SX	40 g	07/05/2013	Broad leaf weeds
	Herbicide	Axial	0.3L	07/05/2013	Wild oats
	Herbicide	Adigor	1.0L	07/05/2013	Wild oats
	PGR	CCC 750	2.0L	19/04/2013	
	PGR	Torpedo	0.1% wv	19/04/2013	
2014	Pesticide	Metarex	4.0 kg	29/10/2013	Slugs
	Herbicide	Lorate	15 g	17/04/2014	Spring weeds
	Herbicide	Starane gold	1.0L	17/04/2014	Spring weeds
	Herbicide	Axial	0.35L	17/04/2014	Wild oats
	Herbicide	Adigor	1.0L	17/04/2014	Wild oats
	PGR	CCC 750	1.75L	17/04/2014	
	PGR	Cutlass	0.20L	17/04/2014	

Selected lines from two doubled-haploid mapping populations were screened over the first two field seasons. In 2011/2012, 39 lines of the CIMMYT Line 14 x Rialto population, 10 lines of the Lynx x Cadenza population and the parent Rialto were included in the field experiment (Table 3.3). L14 x Rialto is a doubled-haploid cross derived from the CIMMYT large ear phenotype spring wheat advanced line 14 (Line 14) and the elite UK winter wheat Rialto. Rialto has been shown to have consistently high radiation-use efficiency (Shearman et al., 2005) and was because of this high source capacity crossed with L14, which has a high sink capacity due to its large ear phenotype. This L14xRialto population has been shown to segregate in previous experiments for source/sink traits (Gaju et al., 2014), such as flag-leaf size and posture, and ear size (rachis length, spikelets ear^{-1} and grains ear^{-1}). The UK wheat cultivar Rialto (released 1995, feed wheat)) contains the

recessive *Vrn-A1* winter allele and L14 and L8 the dominant *Vrn-A1* spring allele on chromosome 5D; similarly L14 and L8 contain the dominant *Ppd-D1a* allele for photoperiod insensitivity on chromosome 2D, whereas Rialto contains the recessive *Ppd-D1b* allele for photoperiod sensitivity. The DH lines were therefore segregating for anthesis date. In these experiments, DH lines of these two populations were selected based on having generally higher yields and later anthesis dates within the ranges for the lines in the populations (i.e. those lines adapted to UK growing conditions) according to previous data in a field experiment at Sutton Bonington with a full fungicide programme (Foulkes, pers.comm).

The Lynx x Cadenza DH lines were selected from a population derived from UK spring wheat (Lynx) and a UK winter wheat (Cadenza) cross developed in John Innes Centre, UK. This population was previously screened for tolerance and a subset of these lines with high/low disease tolerance was used in the field experiments each year as reference lines.

In 2012/2013, six Lynx x Cadenza DH lines were repeated as reference lines. Doubled haploid lines from another CIMMYT spring wheat crossed with Rialto was screened for disease tolerance this year, in total 20 DH genotypes. The CIMMYT spring wheat parent was Line 8, a large ear phenotype spring wheat advanced line. In addition, 14 UK commercial winter wheat cultivars, whose release dates varied from 1980 to 2008, were also included (Table 3.4).

In 2013/2014, 16 DH lines were screened for disease tolerance and phenotyped for physiological traits in more detail. Of these 16 lines, 14 had been used in the previous years: four Lynx x Cadenza lines, eight L14 x Rialto lines and two L8 x Rialto lines. Two new lines were introduced from an Avalon x Cadenza DH mapping population and had previously displayed high disease tolerance (Julie Smith, pers. comm.; Table 3.5).

Table 3.3. Wheat genotypes used in the 2011/2012 field experiment, their genetic background and the groupings according to their development dates.

Name	Background	Group	Rht-B1
Rialto	UK winter wheat	Late	a
L14 x Rialto 5	CIMMYT spring wheat x UK winter wheat	Early	b
L14 x Rialto 7	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 10	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 15	CIMMYT spring wheat x UK winter wheat	Early	*
L14 x Rialto 16	CIMMYT spring wheat x UK winter wheat	Middle	a
L14 x Rialto 19	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 20	CIMMYT spring wheat x UK winter wheat	Late	*
L14 x Rialto 22	CIMMYT spring wheat x UK winter wheat	Middle	a
L14 x Rialto 23	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 35	CIMMYT spring wheat x UK winter wheat	Middle	a
L14 x Rialto 43	CIMMYT spring wheat x UK winter wheat	Early	a
L14 x Rialto 50	CIMMYT spring wheat x UK winter wheat	Early	b
L14 x Rialto 55	CIMMYT spring wheat x UK winter wheat	Middle	a
L14 x Rialto 59	CIMMYT spring wheat x UK winter wheat	Early	b
L14 x Rialto 62	CIMMYT spring wheat x UK winter wheat	Early	b
L14 x Rialto 66	CIMMYT spring wheat x UK winter wheat	Late	b
L14 x Rialto 68	CIMMYT spring wheat x UK winter wheat	Late	a
L14 x Rialto 70	CIMMYT spring wheat x UK winter wheat	Late	b
L14 x Rialto 74	CIMMYT spring wheat x UK winter wheat	Early	b
L14 x Rialto 80	CIMMYT spring wheat x UK winter wheat	Early	b
L14 x Rialto 82	CIMMYT spring wheat x UK winter wheat	Late	*
L14 x Rialto 85	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 89	CIMMYT spring wheat x UK winter wheat	Early	a
L14 x Rialto 91	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 92	CIMMYT spring wheat x UK winter wheat	Middle	a
L14 x Rialto 104	CIMMYT spring wheat x UK winter wheat	Early	a
L14 x Rialto 107	CIMMYT spring wheat x UK winter wheat	Late	b
L14 x Rialto 112	CIMMYT spring wheat x UK winter wheat	Early	b
L14 x Rialto 114	CIMMYT spring wheat x UK winter wheat	Late	a
L14 x Rialto 119	CIMMYT spring wheat x UK winter wheat	Middle	*
L14 x Rialto 121	CIMMYT spring wheat x UK winter wheat	Early	a
L14 x Rialto 123	CIMMYT spring wheat x UK winter wheat	Middle	a
L14 x Rialto 127	CIMMYT spring wheat x UK winter wheat	Middle	a
L14 x Rialto 129	CIMMYT spring wheat x UK winter wheat	Early	a
L14 x Rialto 131	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 133	CIMMYT spring wheat x UK winter wheat	Middle	b
L14 x Rialto 136	CIMMYT spring wheat x UK winter wheat	Late	b
L14 x Rialto 138	CIMMYT spring wheat x UK winter wheat	Early	a
Lynx x Cadenza 7a	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 5H	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 3D	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 43A	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 42C	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 8I	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 48D	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 14B	UK winter wheat x UK spring wheat	Late	a
Lynx x Cadenza 6E	UK winter wheat x UK spring wheat	Middle	a
Lynx x Cadenza 41F	UK winter wheat x UK spring wheat	Late	a

Table 3.4. Wheat genotypes used in the 2012/20143 field experiment, their genetic background, year of release to the UK market (winter wheat varieties only) and the groupings according to their development dates.

Name	Background	Release year	Group
L8 x Rialto 10	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 11	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 14	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 16	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 20	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 28	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 34	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 43	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 44	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 49	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 51	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 54	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 57	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 60	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 64	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 67	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 71	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 74	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 80	CIMMYT spring wheat x UK winter wheat		Early
L8 x Rialto 84	CIMMYT spring wheat x UK winter wheat		Early
Lynx x Cadenza 3D	UK winter wheat x UK spring wheat		Late
Lynx x Cadenza 5H	UK winter wheat x UK spring wheat		Late
Lynx x Cadenza 7A	UK winter wheat x UK spring wheat		Late
Lynx x Cadenza 14B	UK winter wheat x UK spring wheat		Late
Lynx x Cadenza 42C	UK winter wheat x UK spring wheat		Late
Lynx x Cadenza 48D	UK winter wheat x UK spring wheat		Late
Rialto	UK winter wheat	1993	Late
Badger	UK winter wheat	1973	Late
Consort	UK winter wheat	1993	Late
Xi19	UK winter wheat	2002	Late
Buster	UK winter wheat	1992	Late
Oakley	UK winter wheat	2008	Late
Battalion	UK winter wheat	2006	Late
Moulin	UK winter wheat	1985	Late
Option	UK winter wheat	2001	Late
Savannah	UK winter wheat	1998	Late
Avalon	UK winter wheat	1980	Early
Claire	UK winter wheat	1999	Late
Charger	UK winter wheat	1996	Late
Malacca	UK winter wheat	1997	Late

Table 3.5. The lines used in the 2013/2014 field experiment and their genetic background.

Name	Background
Avalon x Cadenza 117	UK winter wheat x UK spring wheat
Avalon x Cadenza 21	UK winter wheat x UK spring wheat
Cadenza x Lynx 14B	UK spring wheat x UK winter wheat
Cadenza x Lynx 43A	UK spring wheat x UK winter wheat
Cadenza x Lynx 5H	UK spring wheat x UK winter wheat
Cadenza x Lynx 7A	UK spring wheat x UK winter wheat
L14 x Rialto 114	CIMMYT spring wheat x UK winter wheat
L14 x Rialto 115	CIMMYT spring wheat x UK winter wheat
L14 x Rialto 116	CIMMYT spring wheat x UK winter wheat
L14 x Rialto 117	CIMMYT spring wheat x UK winter wheat
L14 x Rialto 118	CIMMYT spring wheat x UK winter wheat
L14 x Rialto 119	CIMMYT spring wheat x UK winter wheat
L14 x Rialto 120	CIMMYT spring wheat x UK winter wheat
L14 x Rialto 121	CIMMYT spring wheat x UK winter wheat
L8 x Rialto 10	CIMMYT spring wheat x UK winter wheat
L8 x Rialto 86	CIMMYT spring wheat x UK winter wheat

3.2.3. Disease and leaf green area assessments

Septoria tritici blotch severity and green leaf area percentages were assessed on three occasions in each field experiment; at growth stage (GS) 59, GS59 + 14 days and at GS59 + 28 days. All live leaf layers (more than 15 % green area) were scored. In 2012 and 2013, ten fertile shoots in each sub-plot were randomly selected for visual assessment. Disease and green leaf area were expressed as a percentage of the total leaf area.

Disease severity was assessed as necrosis associated with disease lesions according to percentage of total leaf area. Only lesions containing pycnidia were considered as true disease lesions. Chlorosis and necrotic areas that did not contain pycnidia were not included in the disease assessment, but were considered in the green leaf area percentage assessments. Figures 3.1a and 3.1b illustrate STB disease in glasshouse experiments and field experiments respectively. In both these cases only necrotic lesions containing pycnidia were assessed as disease. Prior to disease assessments, the Distrain computer program was used for training of estimating disease percentages (Tomerlin and Howell, 1988).

a



b



Fig 3.1. Examples of diseased leaves from a) glasshouse experiment and b) 2012 field experiment.

Leaf area was measured using a leaf form factor, in which the length and width (at the widest point) of each leaf were measured and the area was calculated using the formula *leaf length * leaf width * 0.83* (Bryson et al., 1997). Due to low plant establishment in the 2013/2014 field experiment, disease assessments, green leaf area scores and leaf measurements were performed on 5 fertile shoots per sub-plot.

Stem-base diseases were assessed on 25 fertile shoots per sub-plot during grain filling at the early dough stage (GS83) in 2012, but not in 2013 and 2014 due to low incidence. Eyespot and sharp eyespot symptoms were assessed on a scale of 0-3. In 2012, Fusarium head blight severity was assessed as percentage of ear affected, with the exception of one replicate where sub-plot values were determined on a 0-10 scale. In 2014, yellow stripe rust was also assessed as a percentage of leaf lamina affected when present.

3.2.3. Crop measurements

3.2.3.1 Crop height, developmental stages and shoot number

Key crop developmental stages were determined by the Zadoks' growth stage scale (Zadok et al., 1974). In each experiment, crop height was measured for four randomly selected fertile shoots per sub-plot, from the base of the stem to the tip of the ear. These measurements were taking place at approximately growth stage 83 in each field experiment.

Ears m^{-2} were measured at four random places per sub-plot, the ears in a single row were counted along a 0.5 m ruler placed between the rows avoiding the outer rows and ends of the plot. These measurements took place at GS83 in all three field experiments.

3.2.3.2 Intercepted radiation

Fractional interception of photosynthetically active radiation (PAR) was measured at early stem extension (GS32), ear emergence (GS59) and early grain filling (GS73) in 2014, using a Delta-T Sunscan ceptometer (Delta-T instruments, Bury-St-Edmunds, UK). In each sub-plot, five randomly distributed measurements were taken above the canopy and at ground level along the sub-plot (facing the sun) with the ceptometer placed at 45° to the crops rows.

3.2.3.3 Growth analysis and DM partitioning at GS32 and GS63 in 2014

An aboveground biomass measurement was conducted at GS32. A quadrat of 0.5 m^2 was randomly sampled from each plot and brought into the laboratory. Fresh weights were noted and the samples were oven dried at 100°C for 48 h and dry weights recorded.

At GS63, an area of 0.5 m by 8 adjacent rows (row width: 13.5 cm) was sampled from each sub-plot (0.54 m^2). The fresh weight was recorded and growth analysis was performed, as described below.

A 25% subsample was separated from the total sample and dried at 100°C for 48 h, after which the dry weight was recorded. Then the fertile shoots were separated from the infertile or dead shoots in the subsample. The infertile (newest fully expanded leaf senesced)/dead (no green area) shoots were counted and dried (100°C, 48h) and the dry weights were recorded. The fertile shoots from the 25% subsample were separated into stems (including leaf sheaths), dead leaf lamina, green leaf lamina and ears. Each component was dried (100°C, 48h) and the weight recorded.

3.2.3.4 Water soluble carbohydrate (WSC) assessment

Ten fertile shoots (those with an ear) per sub-plot were randomly sampled between 11.00 and 14.00 h at GS59 + 7 days and at physiological maturity. The ears of these shoots were removed at the collar and the leaves at the ligules, and fresh weights were recorded. The samples were oven dried at 100°C for 24h, and the dry weights were recorded. The samples were submitted for WSC determination by the ‘anthrone’ method, as described by

Yemm and Willis (1954) and following specific procedures described in Galicia et al. (2009). The WSC content amounts were calculated from WSC (%) according to equation 3.1 and the stem WSC content in g m^{-2} was calculated by multiplying stem WSC (g $stem^{-1}$) by fertile shoot m^{-2} at GS63.

Eq 3.1 $$WSC\ (g\ stem^{-1}) = \frac{WSC\ (\%)}{100} \cdot DM\ fertile\ shoots^{-1}$$

3.2.3.5 Harvest measurements

The mean area harvested per sub-plot in each year was 18.7 m^2 in 2012 and 2013, and 17.6 m^2 in 2014.

Thousand grain weight and moisture content of subsamples of the harvested grain from each plot was measured. The moisture contents were determined using a GAC 2000 (Dickey-John Corp., USA. The plot grain yields were expressed as tonnes per hectare at 85% dry matter.

Pre-harvest, the above ground biomass of 50 fertile shoots per plot was measured. The shoots were separated into ears and straw, and the fresh weights were recorded. These were then dried at 100°C for 48 h, after which the dry weight was recorded.

3.2.5. Leaf sample collection for molecular analysis

In each experiment, flag leaves assessed for visual disease severity were also sampled for molecular disease quantification. If the leaf was considered too diseased for an accurate disease assessment (i.e. fully senesced or more than 75% disease), it was discarded. In 2012, ten leaves per leaf layer were sampled by three cores (0.2 cm^2 each) per leaf. The cores were taken evenly distributed along the leaf. All leaf cores of a leaf layer were collected in a 2 mL micro centrifuge tube, fresh weight was recorded and the sample was stored at -20°C until freeze-drying could take place. In 2014, five whole leaves per plot were weighed and sampled in a 50 mL falcon tube directly after disease assessments were performed, and stored at -20°C.

3.3 Glasshouse experiments

3.3.1. Plant material and growth conditions

The seed used in the glasshouse experiments were germinated in Petri dishes prior to planting. The Petri dishes were lined with filter paper, flooded with sterilised tap water and the seed were added to the Petri dishes.

The Petri dishes were covered with parafilm and incubated in darkness at 18°C for 4-5 days. Five seeds per pot were planted in 11 x 11 cm pots filled with peat-based potting mixture (Bord na Móna, Ireland). Only pre-germinated seeds with fully emerged coleoptiles were used in the experiments. The plants were grown in glasshouse conditions of 16 h daylight at 18°C and 8 h dark at 16°C for two weeks, watered as needed every 2-3 days until the second leaf was developed. The plants of the first two glasshouse experiments (the "inoculum concentration experiments") were grown in the same conditions after inoculations. The plants in the third experiment ("variety experiment") were grown in natural daylight conditions after inoculation, about 12 h daylight (at 16-19 °C) and night time temperatures between 10 and 13°C. In the inoculum concentration experiments, two winter wheat cultivars were used: Rialto and Xi19. In the third experiment ("variety experiment") ten genotypes were used based on contrasting backgrounds and resistance (Table 3.6).

Table 3.6. Details of genotypes selected for the 'cultivar' glasshouse experiment. The most recent available resistance rating for each variety is reported, and when possible the Irish resistance rating was selected.

Genotype	Resistance rating	Parent 1	Parent 2
L14 x R127	n/a	Rialto	Line 14
CxL 5H	n/a	Lynx	Cadenza
Xi19	5[1]	Cadenza x Rialto	Cadenza
Rialto	5[2]	Haven	Fresco
Cordiale	4[2]	Reaper x Cadenza	Malacca
JB Diego	5[2]	3351b	Stru2374
Alchemy	6[1]	Claire	Consort x Woodstock
Robigus	5[1]	Z-836	Putch
Stigg	7[1]	Biscay x LW-96-2930	Tanker
Gallant	4[1]	Malacca x Charger	Xi19

1) HGCA (AHDB) recommended list
2) Department of Agriculture, Food and the Marine, Ireland recommended list

3.3.2 Pathogen growth and inoculation

Sixteen unidentified *Z. tritici* isolates collected from Teagasc Oak Park field experiments in 2013 were cultivated on potato dextrose agar for 4-5 days (in darkness at 18°C) by spotting 30 µL of glycerol spore suspension stock on plates. Following incubation these were transferred onto new plates in order to bulk them up, this time spread over entire plates. The *Z. tritici* spores were collected by flooding the cultures with distilled H_2O and gently scraping the spores off the plates. The spore-solutions were bulked together in a single

bottle and the concentration of spores ml^{-1} determined using a plastic slide (Kova International, USA). The spore solutions were diluted to desired concentrations (see section 3.3.3) with distilled H_2O and 2-3 large drops of Tween 20 (Sigma-Aldrich, USA) was added. The spore solutions were sprayed on the seedlings until run-off, and the pots were covered with plastic bags for 72 h. The plastic bags were then removed. The spore collections and inoculations were performed in a single day in all experiments. The leaves above the second leaf were trimmed every 3 days until the end of each experiment.

3.3.3 Experimental design

The first two glasshouse experiments were designed as semi-randomised block designs with four replicates. Within each replicate, the contrasting inoculation concentration treatments were separated to prevent cross contamination. Within each concentration level, the sampling dates x genotype combinations were fully randomised. The inoculation concentration treatments were spore solutions of 10^4, 10^5 and 10^6 ml^{-1} and a control treatment (Sterilised Distilled Water) and these were sampled at 0, 7, 14, 21 and 28 days post inoculation. The exception was for the second experiment which was not sampled at 28 days. The inoculation took place based on increasing inoculum concentration, i.e. all control pots were inoculated, followed by the pots inoculated by the lowest concentration of inoculum.

The third glasshouse experiment (the “variety experiment”) was set up as a split-plot design with inoculation treatment as main plots and the genotype x sampling date combinations randomised as sub-plots. This experiment contained six replicates and two inoculation levels, a spore solution of 10^6 ml^{-1} and a control treatment (autoclaved H_2O). Two samplings took place, at 28 and 35 days post inoculation. This experiment was performed using separate inoculum sources for each of the six replicates. Each *Z. tritici* isolate was cultivated on separate plates for each block and each block was inoculated using a separate inoculum solution.

3.3.3 Assessments and sampling

The second leaf of each plant in a pot (5 leaves in total) was collected and assessed for percentage visual disease. The assessed leaves of each pot were bulked into a single 50 mL falcon tube and stored at -20°C until freeze-dried for 36h. The dried samples were transferred into 2 mL eppindorf tubes.

Glass beads (5 mm in diameter) were added to the tubes and the samples were homogenised in a mixer mill at 20 shakes second^{-1} for 8 minutes in preparation for DNA extraction.

3.4 Molecular assays for *Z. tritici* pathogen load

3.4.1 Bioinformatics

Published gene nucleotide sequences were obtained by the NCBI database (www.ncbi.nlm.nih.gov). Primer and probe specificity were determined using the primer-BLAST tool on the NCBI website. Sequence alignments were performed on the online tool ClustalW2 (www.ebi.ac.uk). Genome sizes were calculated using equation 3.X (Ronning et al., 2006), based on estimations by Goodwin et al. (2011) and Brenchley et al. (2012) for *Z. tritici* and wheat respectively.

Eq. 3.2 $$m = n(1.096 \times 10^{-21})$$

m = mass (g)

n = genome size (no. of nucleotides)

3.4.2 DNA extractions

DNA extractions were performed on leaf samples collected from both the glasshouse and field experiments. All samples were freeze-dried for 48h (Labconco, USA), and homogenised using a mixer-mill (MM400, Retsch, Germany) at 20 shakes second^{-1} for 8 minutes. The full samples of the glasshouse leaves (approx. 70 mg) and field samples collected in 2012 (approx. 20 mg) were extracted. Due to a large sample size, 70 mg subsamples of the 2014 field samples were extracted.

All glasshouse and field samples were extracted using a CTAB protocol, except samples collected at GS59+14d in 2012. These samples were extracted by a GenElute Plant Genomic DNA miniprep kit (Sigma-Aldrich, USA), according to manufacturer's instructions. The DNA extractions using the CTAB method were loosely based on the method developed by Zhang and Stewart (2000) for samples containing large amounts of secondary metabolites, in combination with the standard procedure of the Teagasc laboratories, as follows below.

500 µL of extraction buffer (0.1M Tris, 1M NaCl, 0.02M EDTA, 2% CTAB, 2% PVP-40, 0.5% 1,1 phenanthroline monohydrate, 0.2% 2-mercaptoethanol) was added to each of the samples, which were then incubated for 30 minutes at 65°C. An equal volume of 1:24 chloroform: isoamyl alcohol was added and the samples were centrifuged at 12,000 x g for 10 minutes at 4°C. The aqueous phase was collected and the same step was repeated again, with 400 µL of the chloroform: isoamyl alcohol solution. The aqueous phase was collected, 500 µL ice-cold 99% isopropanol added and the samples were chilled at -20°C for 2h. The samples were centrifuged at 12,000 x g for 15 minutes at 4°C, and the pellets were washed with 70% ethanol with 5 min centrifugation at 12,000 x g and this was then repeated with 100% ethanol. The pellets were air dried in a laminar flow hood and re-suspended with 200 µL TE-buffer.

For the field leaf samples that yielded clean, white and easily re-suspended pellets, in the extraction process the samples were immediately treated with 3 µL RNAse (10 mg mL^{-1}). For the remaining samples, an additional cleaning step was performed. 500 µL of cleaning buffer (0.05M Tris, 0.05M EDTA, 2% CTAB, 2 % NaCl, 0.02% 1,1 phenanthroline monohydrate) were added into the DNA solutions and the samples were gently shaken at room temperature for 1 hour. The samples were re-pelleted by centrifugation at 12,000 x g for 5 min at 4°C, and the pellet was washed with an ethanol solution (80% EtOH, 15 mM ammonium acetate) and finally with 100% ethanol. The pellet was air dried, re-suspended and RNAse treated as described above.

The total DNA concentration was quantified by NanoDrop 2000 (Thermo Scientific, USA), according to manufacturer's instructions. The glasshouse samples were also quantified by gel electrophoresis using DNA standards prepared with lambda DNA (New England Biolabs, USA). 1 µL of sample was mixed with 6X loading dye (manufacturer) and sterile H_20. The samples were loaded onto an 1% agarose gel (0.01% EtBr) at 85V and visualised using Image Station 440cf transilluminator (Kodak Digital Systems, USA). The reference samples for the Real-Time assay were quantified using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, USA) in the LightCycler 480. The reaction was performed according to the manufacturer's instructions except that the volume of the Picogreen was modified to 100 µL per reaction. The quantification was performed using a 465-510 nm filter and the following

cycling conditions; 20°C for 10s, 5 cycles of 37°C for 10s with a single acquisition and finally 20°C for 10s.

3.4.3 Real-Time PCR

Targets

A *Z. tritici* primer/probe combination was chosen from Bearchell et al. (2005) and amplifies a 63 base pair (bp) sequence in the ***β-tubulin*** gene (Table 3.6). The wheat target was selected from Ronning et al. (2006) and amplifies a 87 bp sequence of the PKABA1 gene located in the wheat nuclear genome (Table 3.6).

Table 3.7. Probes and primers used in the Real-Time PCR quantifications. The *Z. tritici* target was the β-tubulin gene (Accession no. AY547264) and the wheat target the PKABA1 gene (Accession no. M94726). The final concentration in the reaction mixture is reported for each primer and probe.

Target	Function	Sequence	Size	Conc.
Z. tritici	probe	5'-FAM-TTACGCCAAGACATTC-MGBNFQ-3'	16 bp	100 nM
	sense	5'-GCCTTCCTACCCCACCATGT-3'	20 bp	400 nM
	antisense	5'-CCTGAATCGCGCATCGTTA-3'	19 bp	400 nM
Wheat	probe	5'-VIC-TCGCACCTCGGCT-MGBNFQ-3'	13 bp	100 nM
	sense	5'-CAAGTATGTCATAGAGATTTGAA-3'	23 bp	800 nM
	antisense	5'-GTAACCGAAGTCACAAATCT-3'	20 bp	800 nM

All Real-Time quantifications were performed using a LightCycler 480 system (Roche, Switzerland) and analysed using LightCycler 480 version 1.5. TaqMan Fast Advanced Master Mix (Applied Biosystems, USA) was used for the β-tubulin – ATP synthase quantification, and Brilliant Multiplex qPCR Master Mix (Agilent Technologies, USA) was used for the β-tubulin – PKABA1 quantification. The cycling conditions were performed according to the respective manufactures instructions with an annealing temperature of 60°C. Primers and probes were added to the mixture as described in Table 3.7.

The quantified reference samples were used to produce standard curves. To standardise *Z. tritici*, isolated genomic DNA from Z. tritici isolate 560.11 was used, diluted in 10-fold serial dilutions with molecular grade H_2O (Sigma-Aldrich, USA). Finally for the wheat PKABA1 gene, wheat genomic DNA isolated from second leaf of Rialto grown in controlled environment was used. The wheat genomic sample was diluted using three-fold serial dilutions. Standard curves were created for each reaction, first separately and then as

multiplex reactions to ensure that the there was no interference between targets.

3.5 Data analysis and calculations

All data processing and calculations below were carried out using Microsoft Excel 2010.

3.4.1 Green lamina index and healthy area duration

The green lamina area index (GLAI) was calculated over the top four leaves according to the formula below (Eq. 3.3) where LA_i is leaf area of leaf layer *i*, *EM* is ears m^{-2}, GLA_i is the green leaf area percentage at leaf layer *i* and *n* is the number of leaf layers. The total leaf area index was calculated using the same formula, except for the exclusion of GLA_i.

Eq. 3.3 $$GLAI = \sum_i^n [LA_i \; x \; EM \; x \; GLA_i]$$

Healthy area duration (HAD) from GS59 to physiological maturity was calculated over the top four leaves at from GLAI assessed at GS59, GS59 + 14 days, GS59 + 28 days and physiological maturity using equation 3.4 where $GLAI_i$ is the green lamina area index at observation *i*, t_i is the number of days between observations and *n* is the number of observations (Parker et al., 2004).

Eq. 3.4 $$HAD = \sum_i^{n-1} \frac{1}{2} [(GLAI_i + GLAI_{i+1})(t_{i+1} - t_i)]$$

Area under disease tolerance curve (AUDPC) was calculated by Eq. 3.4, substituting GLAI for percentage STB symptoms on the flag leaf layer. Integrated pathogen load was also calculated by this equation, by substituting GLAI for pg STB DNA ng wheat DNA^{-1}.

Disease tolerance was calculated from the slope of a linear regression, with HAD as the explanatory variate and grain yield as the response variate. A steep slope between HAD and yield (and a more negative slope value) indicates disease intolerance, while a more planar slope (less negative slope value) indicates higher disease tolerance. In chapter 7, disease tolerance is also calculated from AUDPC and integrated pathogen load as explanatory variates in the regression analysis.

3.4.2 Radiation interception and radiation use efficiency

The fractional radiation interception (RI) was calculated at growth stages 32, 59 and 73 in 2014, using equation 3.5 where *I* is intermittent PAR and I_0 is below canopy PAR.

Eq. 3.5. $$RI = \frac{I - I_0}{I}$$

Pre-anthesis radiation-use efficiency was calculated on the basis of above-ground dry matter at GS32 and GS63. Photosynthetically active radiation (PAR) was estimated as half of the total radiation, and the accumulated PAR interception was calculated by the interpolated fractional radiation interception based on ceptometer measurements at GS32 and GS59 and the daily incoming radiation (metoffice.gov.uk). The radiation-use efficiency was calculated according to equation 3.6 where the differences between accumulated biomass and accumulated PAR was calculated between observation *i* the following observation (*i+1*).

Eq. 3.6 $$RUE = \frac{Biomass_{i+1} - Biomass_i}{Acc.PAR_{i+1} - Acc.PAR_i}$$

Post-anthesis radiation-use efficiency was calculated using GLAI and the extinction coefficient *k*. GLAI was calculated at growth stage 59, 59+14, 59+28 and 59+ 36, and the GLAI values were interpolated between these until physiological maturity. The *k* value was calculated at GS59 using equation 3.7 where *I* is intermittent PAR and I_0 is below canopy PAR (Bingham et al., 2007).

Eq. 3.7. $$k = \frac{\ln(\frac{I}{I_0})}{GLAI}$$

The fraction intercepted PAR at each date was calculated using *Beer's law* (Eq. 3.8), and the post-anthesis RUE was finally calculated using equation 3.6.

Eq. 3.8. $$1 - exp^{-k \cdot GLAI}$$

3.6 Statistical analyses

All statistical analyses were carried out using GenStat 14th edition. Analysis of variance (ANOVA) was used to determine if there were differences between genotype groups. As the field experiments were designed as split-

plot designs, split-plot ANOVAs with replicates were used for all field traits in which both treatments were analysed. In these analyses, fungicide treatments were main plots and genotypes as sub-plots. Traits that were only studied in one of the fungicide treatments, for instance specific leaf weight, were analysed using one-way ANOVA with replicate structure. The cross-year analysis was performed using an ANOVA, with year, fungicide and cultivar treatment considered as random effects and replicates as fixed effects. Treatment means were compared using the LSD of the means of Fisher, calculated from standard errors of the difference of the means using appropriate degrees of freedom, when the ANOVA indicated significant differences.

To determine relationships between traits, linear regression analyses were performed on sub-plot or sample values (as opposed to genotype means). In order to determine covariate effects on some of the relationships determined by regression analyses, Analysis of Covariance was performed. This analysis first determines the overall relationship by fitting a single linear regression through the dataset. After this, the covariate regression intercepts were allowed to vary (i.e. a parallel regression), and finally both the intercepts and slopes of the covariate regressions were allowed to vary.

Boxplots were used to display spreads in trait values between genotypes. The values presented in the boxplots are defined in Fig 3.2.

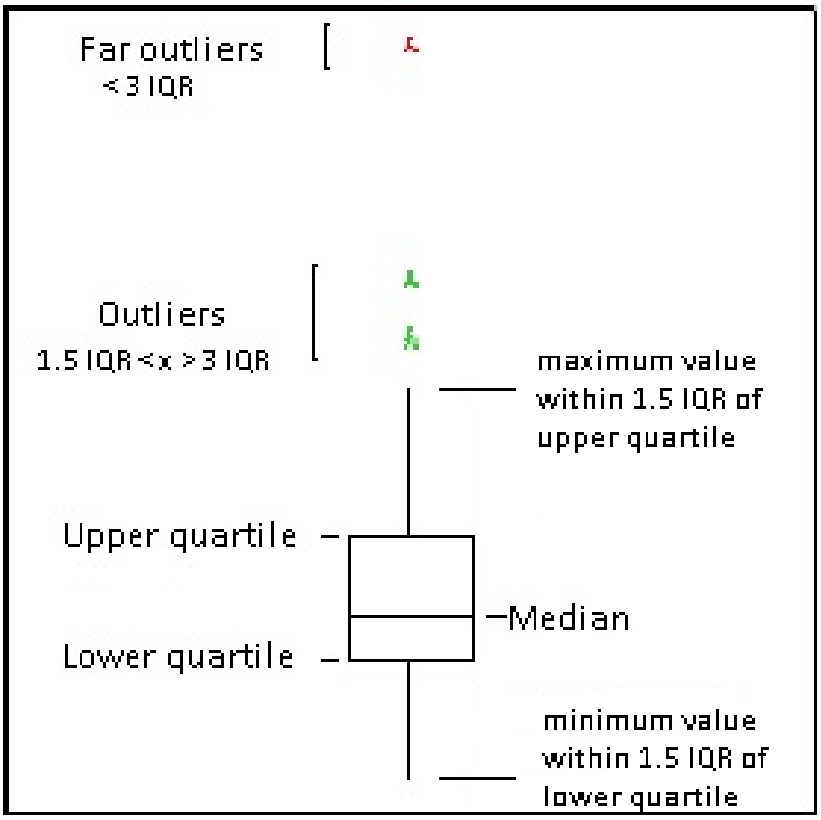


Fig 3.2. Values presented in the boxplots. IQR: Interquartile range (upper quartile – lower quartile). Green outliers are values between 1.5 and 3 interquartile ranges, and the far outliers in red are values over 3 IQR.

4. Quantifying genetic variation in disease tolerance

4.1 Introduction

Disease tolerance is the ability to maintain grain yield potential in the presence of disease symptoms (Parker et al., 2004). Variation in grain yield response to disease has been observed since the late 19th century (Schafer, 1971) but it remains the least well-known self-defence mechanism, compared to disease resistance and escape. Genetic variation in disease tolerance has been observed in several cereal crops, for instance, wheat, oats and barley (Parker et al., 2004, Newton and Thomas, 1994, Politowski and Browning, 1978), but it is still not certain which traits confer tolerance. Studies have shown that a major limitation of disease tolerance is its apparent association with low yield potential (Bingham et al., 2009).

Disease tolerance for a given genotype is quantified by the linear slope between disease symptoms and grain yield. The disease symptoms are assessed throughout the season and are expressed as an integrated area under the disease symptom curve function (Bingham et al., 2009). Traditionally, an area under disease progress curve (AUDPC) has been used to calculate tolerance, but more recently it has been reported that using healthy area duration (HAD), i.e. integrated area under the green lamina area curve, might decrease the effects of site and season variation (Bingham et al., 2009). Using HAD to calculate tolerance it has been shown that there are significant, but limited, differences in disease tolerance in UK adapted wheat varieties (Parker et al., 2004). As there is apparently relatively little genetic variation in wheat varieties developed for north-western Europe, introducing genetic variability from wider wheat germplasm could increase the range of disease tolerance.

In this study, disease tolerance of Septoria tritici blotch (STB) was quantified in lines of two doubled-haploid (DH) populations derived from crosses between the UK winter wheat variety Rialto and two CIMMYT spring wheat advanced lines Line 14 and Line 8 (Gaju et al., 2009). The L14/L8 x Rialto populations were selected as they segregate for a number of source/sink traits, including leaf size and angle, number of tillers per plant, spikelets per ear and grains per ear, which could potentially influence disease

tolerance (Bingham et al., 2009). In addition, the CIMMYT parental lines (L14 and L8) were selected in the CIMMYT shuttle plant breeding program in Mexico. In this program wheat is selected for grain yield in alternate generations with and without fungicides (hence, in the presence of disease in several generations), and should as a consequence display increased disease tolerance compared to UK varieties. In order to determine the actual difference in disease tolerance in the novel DH lines compared to UK adapted material each year, selected UK adapted winter wheat Cadenza x Lynx DH lines with known levels of disease tolerance were included as reference lines each year. In the third year two lines from the Avalon x Cadenza DH winter wheat population were also included due to previous evidence of high disease tolerance.

The specific hypotheses tested in this chapter are:

1) The doubled-haploid lines derived from crosses between Rialto and CIMMYT spring wheat advanced lines of large ear phenotype have increased disease tolerance compared to UK adapted material.
2) Disease tolerance in wheat is heritable and will be consistently expressed in genotypes over sites and seasons.
3) It is possible to identify variation in disease tolerance amongst genotypes independent of grain yield potential.

4.2 Materials and methods

Three field experiments were performed; in 2011-12, 2012-13 and 2013-14. In this chapter, and the following chapters, these site-seasons will be referred to as years. The first two experiments (2012 and 2013) were conducted at the Teagasc experimental station in Carlow, Ireland, and the third experiment (2014) at ADAS Rosemaund, Herefordshire, UK. The experiments used split-plot designs with two fungicide treatments; fully protected and untreated against STB (referred to as “protected” and “untreated” throughout the chapter). The fungicide treatments were randomised on main plots, and genotypes randomised on sub-plots. Each treatment combination was replicated three times. Dates of sowing, seed rates, fungicide applications etc. are detailed in Chapter 3.

In 2012, 39 doubled-haploid L14 x Rialto genotypes were screened for *Z. tritici* in combination with 10 reference genotypes (Cadenza x Lynx DH

genotypes) and the parent Rialto. In 2013, 20 genotypes from the L8 x Rialto DH population were screened, with six Cadenza x Lynx DH reference lines, and 14 commercial winter wheat varieties including the parent Rialto. In the final field experiment in 2014, 16 genotypes of high/low disease tolerance were screened. Out of these, 14 genotypes (8 lines of L14 x R, 2 lines of L8xR and 4 lines of C x L) had been screened in the previous two years and in addition, two Avalon x Cadenza DH lines were included.

Dates of development stages (ear emergence GS59, and anthesis, GS61) were recorded in each sub-plot (Zadok et al., 1974). Disease assessments for Septoria tritici blotch were conducted on three occasions; at GS59, GS59 + 14 days, and GS59 + 28 days. Ten fertile shoots in each plot were visually assessed for percentage STB symptoms and green leaf area on all live leaf layers, and total leaf areas were measured using the FLAME method (Bryson et al., 1997). Green leaf area index (GLAI) was calculated over the top four leaf layers based on FLAME measurements at GS59. Healthy area duration (HAD) was calculated based on GLAI at the three assessment dates and at physiological maturity, integrated over time (see Chapter 3). Crop height to the tip of the ear and ears m^{-2} (in a single row using a 0.5 m ruler placed between rows) were measured at four locations in each plot, at approximately GS85. Sub-plots were machine-harvested to measure grain yield and post-harvest analyses included grains per m^2, thousand grain weight and grain moisture content (see Chapter 3 for full details).

4.3 Results

4.3.1 Weather data

The weather conditions varied considerably during the three field experiments. The winter of 2012 (January – March) was warm and dry with a mean temperature of 7.3°C, compared to the long term mean (LTM) of 6.1°C (Table 4.1). The temperature during the stem-extension period (April - May) was cool, about 1.2°C lower than the LTM, with average rainfall (Table 4.1). The grain filling period (June – July) was cooler than the LTM and high rainfall occurred (239 mm, compared to the LTM 120 mm, Table 4.1).

The winter of 2013 (January – March) was considerably colder than 2012 with an average temperature of 4.6°C, significantly below the LTM of 6.1 °C (Fig 4.1), and lower than average rainfall (Fig 4.2). During stem extension

(April - May) temperatures were similar to 2012 but there was less rainfall (80 mm compared to 106.5 mm, Table 4.1). The grain filling period in 2013 was dry (70 mm rainfall, LTM 120 mm, Table 4.1) and warm particularly in July with an average temperature 2°C higher than the LTM (Fig 4.1).

2014 began with warmer than average temperatures (January – March, Fig 4.1, Table 4.1). There was high rainfall (321 mm) compared to the LTM of 194 mm (Fig 4.2, Table 4.1). Similarly, during stem-extension (April - May) temperatures were warmer than the LTM with higher than average rainfall (139 mm compared to 116 mm LTM). The grain-filling period (June-July) was warmer and slightly drier than the long-term mean (Table 4.1).

Table 4.1. Overview of mean temperatures, total rainfall and long-term means (LTM) of the site-seasons. The weather data are presented according to approximate crop developmental phases (foundation, stem extension and grain-filling).

2012	**Oak Park**					
Season	**Crop Stage**	**Months**	Temp. (°C)	Rainfall (mm)	Temp. LTM (°C)	Rainfall LTM (mm)
Winter	Foundation	Jan-Mar	7.3	113.3	6.1	201.1
Spring	Stem ext.	Apr-May	9.0	106.5	10.2	115.7
Summer	Grain-fill.	June-July	13.9	239.3	15.1	119.5
2013	**Oak Park**					
Season	**Crop stage**	**Months**	Temp. (°C)	Rainfall (mm)	Temp. LTM (°C)	Rainfall LTM (mm)
Winter	Foundation	Jan-Mar	4.6	169.6	6.1	201.1
Spring	Stem ext.	Apr-May	9.0	80.0	10.2	115.7
Summer	Grain-fill.	June-July	15.9	69.8	15.1	119.5
2014	**Hereford**					
Season	**Crop stage**	**Months**	Temp. (°C)	Rainfall (mm)	Temp. LTM (°C)	Rainfall LTM (mm)
Winter	Foundation	Jan-Mar	6.0	321.4	5.1	193.5
Spring	Stem ext.	Apr-May	10.8	138.6	9.9	116.1
Summer	Grain-fill.	June-July	16	92.1	15.3	112.7

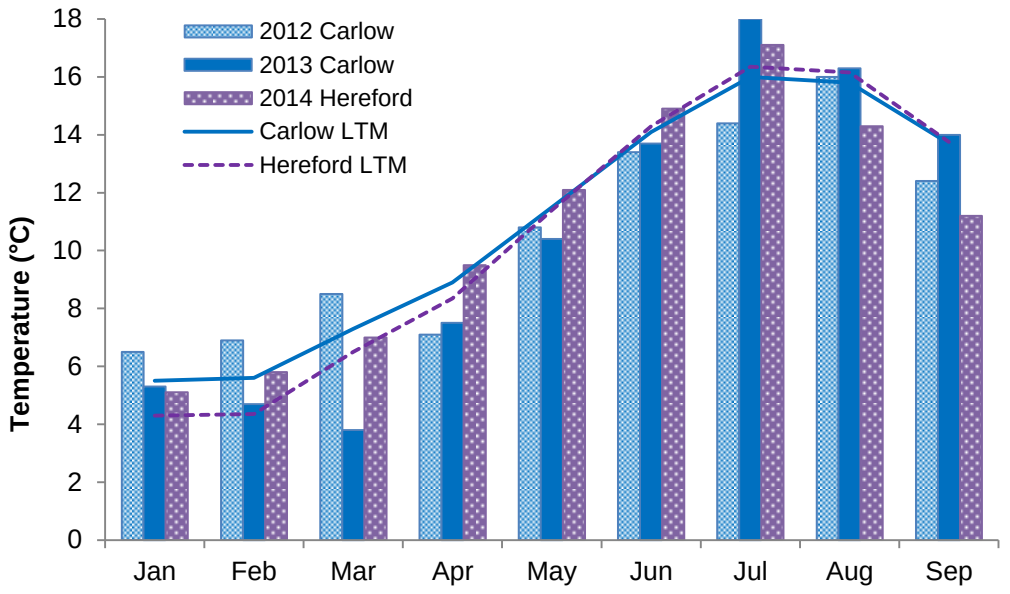


Fig 4.1. Mean monthly temperatures of the field seasons 2011-2, 2012-3, and 2013-4. The long-term mean (LTM) for each site is also displayed. The Carlow data (Oak Park weather station, LTM 2007-2014) were obtained from Met Eirann (www.meteirann.ie 2014), and the Hereford data (Shobdon SAWS weather station, LTM 1981 - 2010) was collected from the Met Office (www.metoffice.gov.uk, 2015).

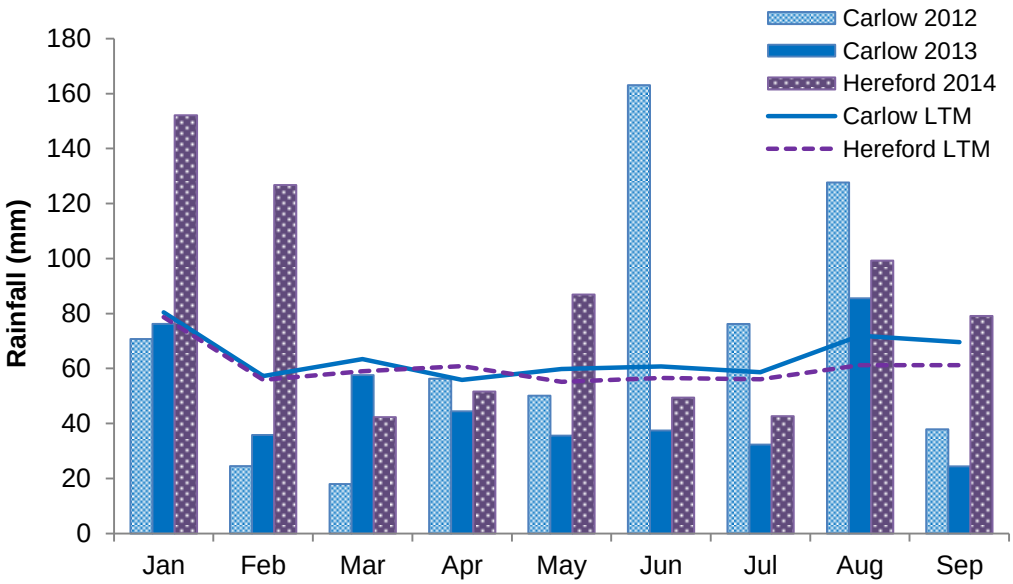
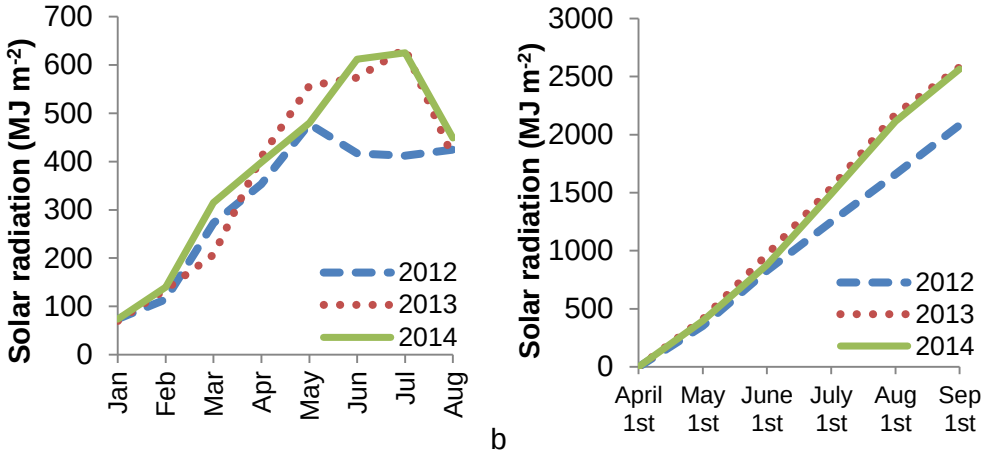


Fig 4.2. Monthly rainfall during the field seasons 2011-2, 2012-3, and 2013-4. The long term mean (LTM) for each site is also displayed. The Carlow data (Oak Park weather station, LTM 2007-2014) were obtained from Met Eirann (www.meteirann.ie 2014), and the Hereford data (Shobdon SAWS weather station, LTM 1981 - 2010) was collected from Met Office (www.metoffice.gov.uk, 2015).

The amount of incident solar radiation during the grain-filling period differed between experiments. In general, both 2013 and 2014 had similar values of solar radiation (Fig 4.3). The 2012 field season had lower values than other years, especially during June and July (Fig 4.3). There was nearly 500 MJ m^{-2} less accumulated radiation during 2012 than the other years (Fig 4.3b).



a b

Fig 4.3. Solar radiation (MJ per m^2) in 2012, 2013 and 2014. A) Monthly average accumulated solar radiation (Jan – Aug) for each field season. B) Accumulated solar radiation from April 1st to September 1st each year. Data obtained from Met Eiràn (www.meteirann.ie, 2015) and Met Office (www.metoffice.gov.uk, 2015)

4.3.2 Crop development and plant height

As a consequence of the variations in weather conditions, there were site-season differences in plant development and also between genotypes in each site-season. In 2012, the earliest genotypes reached ear emergence (GS59) during 24-31 May and the latest during 8-13 June (Table 4.2). In 2013, the earliest genotypes reached ear emergence during 10-15 June and the latest genotype on 24 June (Appendix Table 4.a). In 2014, ear emergence was reached between 5 and 9 June, except for one line that headed on 28 May (Table 4.3). The date of physiological maturity (defined as less than 15% stem area remaining green) also varied between genotypes and site-seasons. In 2012, in the untreated sub-plots physiological maturity occurred from 22-27 July (Fig 4.4, Table 4.2). The protected genotypes reached physiological maturity between 17 July and 7 August. In 2013, drought conditions in July led to physiological maturity occurring in all lines from 22-28 July regardless of fungicide treatment. During 2014, the untreated genotypes reached maturity

from 2-18 July and the protected sub-plots from 10 - 21July (Fig 4.4, Table 4.3).

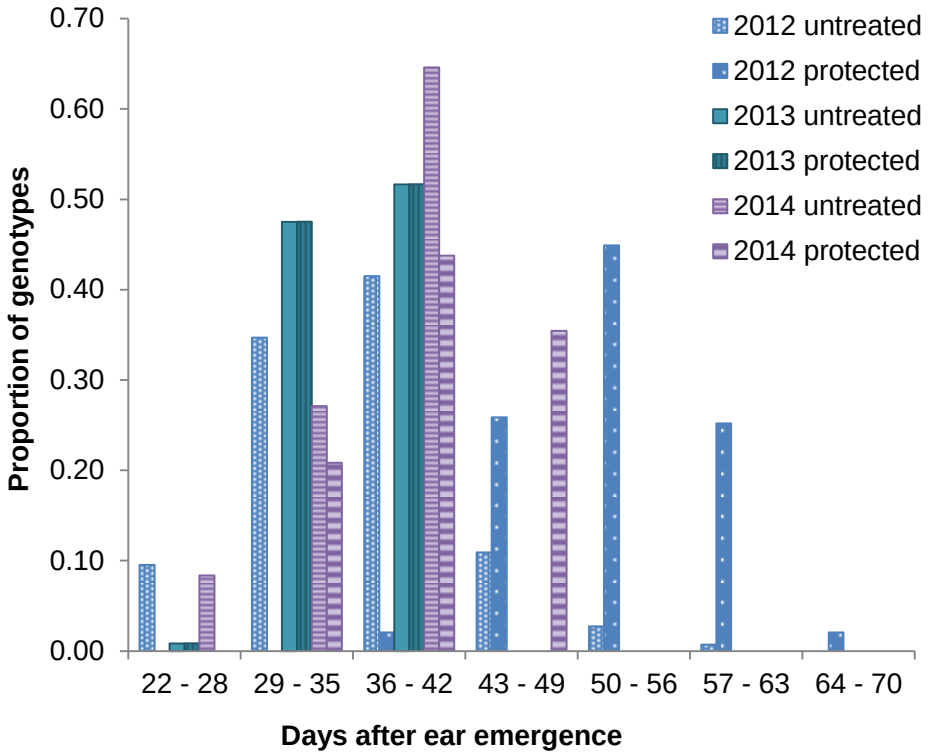


Fig 4.4. Date of physiological maturity in 2011-2, 2012-13 and 2013-4. Proportion of lines of each treatment grouped into seven day intervals after ear emergence (week 4 to week 10).

Crop height was measured shortly before physiological maturity. In 2012, there were differences in height between treatments ($p=0.014$) and genotypes ($p<0.001$) as well as a fungicide treatment (FT) x genotype (G) interaction ($p=0.001$). The untreated plots were slightly taller, with a genotype range of 63.0 – 105.6 cm (avg. 84.2 cm) compared to the fungicide-protected range of 62.8 – 98.5 cm (avg. 79.8 cm; Table 4.2). There were no differences between treatments in 2013, but there was a genotype difference in crop height (63.0 – 115.7 cm; $p<0.001$; Appendix table 4.1). There was no significant FT x G interaction in 2013, even though the untreated crops were slightly taller than the fungicide-protected crops. In 2014, there were again genotypic differences in height in the range 79 – 133 cm ($p<0.001$; Table 4.3), but not between treatments and there was no fungicide treatment x genotype interaction.

Table 4.2. Summary of plant developmental dates and crop heights in 2012.

Genotype	Ear fully emerged (GS5)	Physiological maturity untreated	Physiological maturity protected	Crop height untreated (cm)	Crop height protected (cm)
Rialto	13 Jun	23 - 27 Jul	31 Jul - 03 Aug	87.2	75.8
L14 x R 5	31 May	05 - 11 Jul	27 - 31 Jul	81.4	79.1
L14 x R 7	07 Jun	11 Jul	27 - 31 Jul	70.9	68.8
L14 x R 10	07 Jun	28 Jun - 11 Jul	23 Jul	67.2	63.5
L14 x R 15	31 May	05 - 11 Jul	27 - 31 Jul	80.1	76.1
L14 x R 16	07 Jun	28 Jun - 27 Jul	03 Aug	103.8	95.7
L14 x R 19	07 Jun	28 Jun - 11 Jul	17 - 27 Jul	70.1	69.9
L14 x R 20	13 Jun	17 - 19 Jul	31 Jul - 03 Aug	81.4	80.3
L14 x R 22	07 Jun	23 - 27 Jul	05 - 17 Jul	93.1	88.9
L14 x R 23	07 Jun	28 Jun - 11 Jul	27 - 31 Jul	82.3	78.3
L14 x R 35	07 Jun	11 - 17 Jul	31 Jul - 08 Aug	95.4	89.3
L14 x R 43	31 May	28 Jun - 11 Jul	19 - 27 Jul	91.8	86.3
L14 x R 50	31 May	05 - 11 Jul	27 Jul - 03 Aug	65.3	63.3
L14 x R 55	07 Jun	28 Jun - 17 Jul	27 - 31 Jul	89.8	87.6
L14 x R 59	31 May	11 - 17 Jul	27 - 31 Jul	63.0	62.8
L14 x R 62	31 May	22 Jun - 11 Jul	23 - 27 Jul	69.8	70.2
L14 x R 66	13 Jun	17 Jul -19 Jul	27 Jul - 03 Aug	84.1	83.4
L14 x R 68	13 Jun	10 Jul - 03 Aug	03 - 07 Aug	105.6	98.5
L14 x R 70	13 Jun	05 - 17 Aug	27 - 07 Aug	83.0	80.0
L14 x R 74	31 May	28 Jun - 11 Jul	31 Jul	79.5	73.9
L14 x R 80	31 May	17 Jul	31 Jul	64.4	64.8
L14 x R 82	13 Jun	05 Jul - 17 Jul	27 Jul - 03 Aug	82.6	80.9
L14 x R 85	07 Jun	05 - 17 Jul	27 Jul	73.5	71.3
L14 x R 89	31 May	05 - 11 Jul	23 - 31 Jul	95.8	91.0
L14 x R 91	07 Jun	28 Jun - 11 Jul	23 - 27 Jul	84.6	79.6
L14 x R 92	07 Jun	17 Jul - 27 Jul	27 Jul - 07 Aug	90.8	84.4
L14 x R 104	31 May	28 Jun	19 Jul - 27 Jul	97.8	90.8
L14 x R 107	13 Jun	05 - 17 Jul	27 Jul - 03 Aug	85.7	83.3
L14 x R 112	31 May	05 - 17 Jul	27 Jul	70.3	70.3
L14 x R 114	13 Jun	19 - 23 Jul	31 Jul	92.0	83.5
L14 x R 119	07 Jun	11 - 17 Jul	23 Jul -03 Aug	84.9	81.4
L14 x R 121	31 May	17 - 27 Jul	27 Jul - 03 Aug	88.8	79.1
L14 x R 123	07 Jun	17 Jul	23 - 27 Jul	87.6	83.7
L14 x R 127	07 Jun	17 Jul	17 - 27 Jul	76.3	69.0
L14 x R 129	31 May	28 Jun - 11 Jul	17 - 23 Jul	85.8	84.5
L14 x R 131	07 Jun	28 Jun - 17 Jul	17 - 27 Jul	72.6	76.9
L14 x R 133	07 Jun	05 - 23 Jul	31 Jul - 03 Aug	92.2	87.5
L14 x R 136	13 Jun	11 - 27 Jul	31 Jul - 03 Aug	87.5	80.7
L14 x R 138	31 May	28 Jun - 17 Jul	27 - 31 Jul	83.0	82.1
CxL 7a	13 Jun	05 - 11 Jul	31 Jul - 03 Aug	69.6	65.1
CxL 5H	13 Jun	17 - 27 Jul	31 Jul - 03 Aug	99.8	95.3
CxL 3D	13 Jun	17 - 19 Jul	31 Jul - 03 Aug	94.8	89.8
CxL 43A	13 Jun	17 - 19 Jul	31 Jul - 03 Aug	83.8	76.0
CxL 42C	13 Jun	17 - 27 Jul	27 Jul - 03 Aug	91.0	79.4
CxL 8I	13 Jun	17 - 23 Jul	03 - 07 Aug	91.7	80.5
CxL 48D	13 Jun	17 - 19 Jul	27 - 31 Jul	93.8	83.7
CxL 14B	13 Jun	19 - 27 Jul	03 Aug	99.1	92.3
CxL 6E	07 Jun	05 - 11 Jul	23 - 27 Jul	76.6	73.4
CxL 41F	13 Jun	17 - 19 Jul	27 - 31 Jul	84.3	77.8

Table 4.3. Plant developmental dates and crop height in 2014. All data values are presented as averages of both untreated and protected treatments, with the exception of the date of physiological maturity (ranges in untreated and protected treatments).

Genotype	Ear emergence (GS59)	Anthesis date	Physiological maturity Untreated	Physiological maturity Protected	Crop height (cm)
AxC 117	05 Jun	10 Jun	12 - 15 Jul	16 - 17 Jul	91.3
AxC 21	05 Jun	10 Jun	14 - 18 Jul	21 Jul	82.3
CxL 14B	07 Jun	12 Jun	15 - 16 Jul	18 - 21 Jul	114.4
CxL 43A	07 Jun	12 Jun	14 - 16 Jul	18 - 21 Jul	91.5
CxL 5H	07 Jun	12 Jun	11 - 14 Jul	15 - 17 Jul	110.1
CxL 7A	05 Jun	10 Jun	05 - 09 Jul	19 Jul	81.7
L14xR 114	07 Jun	12 Jun	12 - 16 Jul	15 - 17 Jul	91.2
L14xR 127	05 Jun	10 Jun	05 - 09 Jul	09 - 11 Jul	84.9
L14xR 133	07 Jun	12 Jun	14 - 17 Jul	19 - 21 Jul	97.3
L14xR 16	07 Jun	12 Jun	11 - 14 Jul	18 - 21 Jul	107.8
L14xR 19	28 May	05 Jun	01 - 09 Jul	09 - 11 Jul	79.5
L14xR 20	05 Jun	10 Jun	12 - 15 Jul	17 - 19 Jul	81.9
L14xR 22	07 Jun	12 Jun	01 - 09 Jul	11 - 12 Jul	94.4
L14xR 92	05 Jun	10 Jun	11 - 15 Jul	14 - 15 Jul	91.6
L8xR 10	07 Jun	12 Jun	14 - 16 Jul	16 - 17 Jul	133.4
L8xR 86	07 Jun	12 Jun	11 Jul	11 - 12 Jul	111.8

4.3.3 Septoria tritici blotch severity

In 2012 there was high disease pressure, and particularly so for Septoria tritici blotch (STB). At the first assessment at GS59, there was overall 97% STB incidence on the flag leaf and 100% on the lower leaves in the untreated plots (Appendix Table 4.c). There were differences in STB symptoms between the fungicide treatments at GS59 with mean untreated disease severities of 3.0%, 9.5% and 17.5%, for the flag leaf, second and third leaf, respectively (Fig 4.5, Table 4.4). There were less STB symptoms on the same leaves in the fungicide-protected treatment (0.3%, 1.1% and 3.9%, respectively; Table 4.5). There were differences ($p<0.001$) in STB disease severity between genotypes at all assessments, and there were also fungicide treatment x genotype interactions at all assessments ($p<0.001$, Table 4.4). At GS59 + 14 days there was a mean flag-leaf disease severity of 9.60% and at GS59 + 28 days of 25% (Table 4.4). It should, however, be noted that the STB severity at GS59 + 28 days is an average of the “live flag leaves”, hence the plots with a flag-leaf green area score of less than 15% were not assessed.

Because of this, the real STB severity is probably higher as the 25 plots (out of 147) with the highest disease severity were excluded. The CIMMYT/UK-derived genotypes generally had higher flag-leaf scores at GS59+14d when compared to the UK-derived genotypes; 10.7% compared to 5.9% ($p<0.001$), respectively. The CIMMYT/UK-derived lines also had a larger range of flag-leaf disease at 1.6 – 24.1% and 0.03 – 1.7% for untreated and protected treatments, respectively (FT x G interaction: $p<0.001$) than the UK-derived genotypes with 1.8 – 8.7% and 0.07 - 0.8% for untreated and protected flag-leaves, respectively (FT x G interaction: $p<0.001$).

In contrast to 2012, there was low disease pressure in 2013 due to hot and dry conditions. At the first assessment (GS59) there was an average STB disease incidence of 15% on the flag-leaf in the untreated plots, considerably lower than the comparable value of 97% in 2012 (Appendix table 4.c). There were no significant differences in STB severity between fungicide treatments on flag leaves in any 2013 assessment, but there were some differences in the lower leaf layers (Appendix Table 4.b). Despite the low disease pressure, there were differences between lines and significant FT x G interactions in all leaf layers except for the flag leaf at GS59 and GS59 + 28 days ($P< 0.05$; Appendix Table 4.b).

The STB severity in 2014 was medium to high (Fig. 4.5). There was a 100% STB incidence in the upper canopy (top three leaf layers) at GS59 in the untreated plots, but only 21% incidence on the untreated flag leaves (Appendix Table 4.c). By GS59 + 14 days, the STB incidence on the untreated flag leaves was 100% (Appendix Table 4.c). At GS59, there were no significant differences between fungicide treatments or between lines on the flag leaf layer, and consequently no FT x G interaction. However, on the following two observations there were differences between treatments ($p=0.040$ and $p=0.003$) as well as between genotypes and significant FT x G interactions for the flag leaf ($p<0.001$ and $p=0.012$; Table 4.4). There was no difference in flag-leaf disease severity between the lines with UK background and the CIMMYT L14/8-derived genotypes at GS59+14d, but there were FT x G interactions within the groups ($p<0.001$). The UK (Lynx x Cadenza and Avalon x Cadenza DH lines) genotypes ranged from 0.7 – 8.2% and 0.2 – 1.2% in the untreated and protected treatment, respectively, and the CIMMYT L14/8-derived genotypes (L14 x Rialto and L8 x Rialto DH lines) from 1.3 – 11.3% and 0.1 – 0.9% in the untreated and protected treatment, respectively.

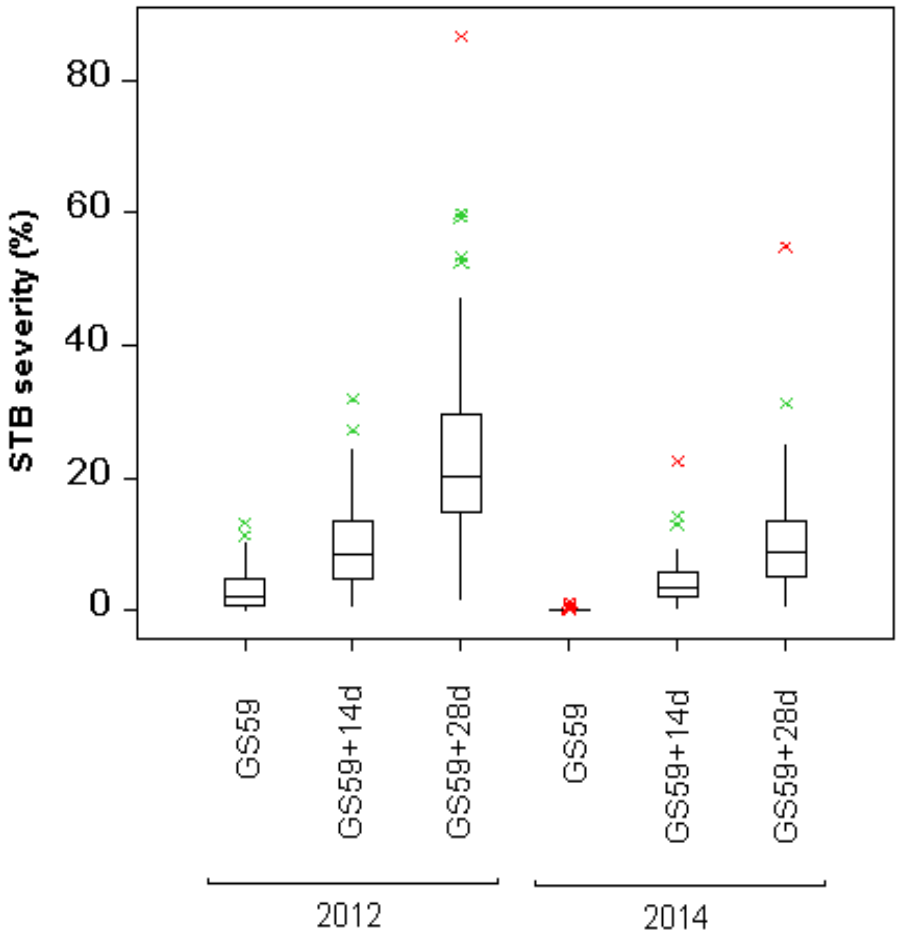


Fig 4.5. Boxplot of Septoria disease severity for 49 genotypes in the 2011/2012 and 16 genotypes in the 2013/2014 seasons. The genotype ranges of disease scored on untreated flag leaves are shown for each assessment at GS59, GS56+14d and GS59+28d. Outliers, more than 1.5 interquartile ranges from the upper quartile, are displayed in green while far outliers (more than 3 interquartile ranges from the upper quartile) are displayed in red.

Table 4.4 Septoria tritici blotch severity**.** Symptoms of Septoria tritici blotch throughout the post-ear emergence period of field seasons 2012 and 2014. Significance limit is p<0.05 and shown in bold font in table, but near-significant values (p<0.10) are included using normal font. The disease severities for the field season 2013 are reported in appendix table 4.2.

Year	Observation	Leaf Layer	Average (%) Untreated	Average (%) Protected	SQRT disease transf. untreated	SQRT disease transf. protected	P treatments SED (df)	P genotypes SED (df)	P G*E interaction SED (df)
2012	GS59	1	3.0	0.3	1.51	0.36	**0.024**	**<0.001**	**<0.001**
							0.180 (2)	0.241 (192)	0.341 (192)
		2	9.5	1.1	2.94	0.78	**0.019**	**<0.001**	**<0.001**
							0.303 (2)	0.269 (192)	0.380 (192)
		3	17.5	3.9	4.12	1.74	**0.015**	**<0.001**	**<0.001**
							0.292 (2)	0.339 (185)	0.479 (185)
	GS59+14 d	1	9.6	0.4	2.95	0.50	**0.005**	**<0.001**	**<0.001**
							0.170 (2)	0.263 (192)	0.372 (192)
		2	20.6	1.6	4.45	1.02	**0.001**	**<0.001**	**<0.001**
							0.119 (2)	0.338 (168)	0.478 (168)
	GS59+28 d	1	25.1	1.7	4.78	1.08	**0.002**	**<0.001**	**<0.001**
							0.166 (2)	0.450 (167)	0.637 (167)
2014	GS59	1	0.1	0.1	0.13	0.19	NS	NS	NS
							*	*	*
		2	1.8	0.4	1.05	0.44	NS	**<0.001**	**0.021**
							*	0.265 (60)	0.375 (60)
		3	11.2	3.3	3.05	1.62	**0.046**	**<0.001**	0.056
							0.316 (2)	0.419 (60)	0.593 (60)
	GS59+14 d	1	4.4	0.9	1.92	0.77	**0.040**	**<0.001**	**<0.001**
							0.235 (2)	0.243 (60)	0.343 (60)
		2	12.6	2.4	3.25	1.41	**0.018**	**<0.001**	**<0.001**
							0.251 (2)	0.334 (60)	0.472 (60)
		3	26.5	9.5	5.09	2.90	**0.013**	**<0.001**	NS
							0.251 (2)	0.473(60)	*
	GS59+28 d	1	10.9	2.4	3.05	1.20	**0.003**	**<0.001**	**0.012**
							0.103 (2)	0.371 (60)	0.524 (60)
		2	28.6	7.8	5.29	2.62	**0.016**	**<0.001**	**<0.001**
							0.343 (2)	0.387(53)	0.547 (53)
		3	43.1	21.7	6.70	4.60	**0.010**	**<0.001**	**0.017**
							0.207 (2)	0.517 (33)	0.732 (33)

The area under disease progress curve (AUDPC) was determined for the flag leaves of the 2012 and 2014 field experiments, over the three assessments. However, due to the high disease pressure in 2012, only a subset (122 out of 147) of the untreated plots could be determined for AUDPC (Table 4.5). In 2012, the average untreated flag leaf AUDPC was 296 % days, which decreased to 20 in the fungicide protected treatment ($p<0.001$). There were large genotype differences ($p<0.001$) and a FT x G interaction ($p=0.014$). In the untreated plots, the AUDPC ranged from 68 to 596 % days and the fungicide protected range was considerably smaller at 3.1 – 51.3. In 2014, AUDPC could be determined for all plots. Due to less disease pressure, the average untreated AUDPC was lower than in 2012 at 118 which then decreased to 25 % days with fungicide protection ($p=0.037$). There was a FT x G interaction ($p=0.027$) and also large differences between genotypes ($p<0.001$), ranging 17 – 346 and 2 – 69 % days in untreated and fungicide protected treatments, respectively (Table 4.6).

In order to determine if disease escape associated with plant height influenced the STB severity, regression analyses between crop height and untreated disease severity at GS59 + 14 days were carried out. There was a weak, negative relationship between STB severity and plant height in 2012 ($p=0.003$, $r^2=0.053$) and a stronger relationship in 2014, where plant height accounted for 19% of the variation in flag-leaf STB severity ($p<0.001$). Similarly, there was no relationship between AUDPC and crop height in 2013, but in 2014 crop height accounted for 19.5 % of the variation in AUDPC ($p<0.001$).

Table 4.5. Area under disease progress curves (AUDPC) in 2012. The average of genotypes within each treatment is reported, and also the number of plots used to calculate the averages. The transformation (logarithm base 10 divided by 10) used for the ANOVA is also reported.

	Untreated			Protected		
Genotype	AUDPC (%days)	AUDPC (log x/10)	Plots	AUDPC (%days)	AUDPC (log x/10)	Plots
L14 x R 10	440.3	1.65	1	51.3	0.76	3
L14 x R 104	471.0	1.67	3	36.9	0.66	3
L14 x R 107	314.1	1.50	2	32.8	0.56	3
L14 x R 112	302.4	1.48	3	31.4	0.58	3
L14 x R 114	223.6	1.36	3	16.8	0.42	3
L14 x R 119	230.0	1.38	3	5.6	0.18	3
L14 x R 121	68.0	0.89	3	6.9	0.21	3
L14 x R 123	148.4	1.18	3	28.5	0.57	3
L14 x R 127	154.3	1.21	3	3.1	0.11	3
L14 x R 129	212.5	1.33	3	11.0	0.29	3
L14 x R 131	172.4	1.26	1	10.0	0.29	3
L14 x R 133	318.5	1.49	3	26.0	0.47	3
L14 x R 136	471.1	1.68	1	26.6	0.48	3
L14 x R 138	357.4	1.56	3	34.7	0.57	3
L14 x R 15	272.2	1.44	3	4.3	0.14	3
L14 x R 16	479.5	1.69	1	43.1	0.72	3
L14 x R 19	451.3	1.63	2	23.6	0.46	3
L14 x R 20	299.6	1.47	3	17.1	0.39	3
L14 x R 22	595.9	1.75	2	25.8	0.55	3
L14 x R 23	419.5	1.63	2	26.1	0.51	3
L14 x R 35	325.5	1.48	3	9.6	0.23	3
L14 x R 43	304.7	1.49	3	6.0	0.18	3
L14 x R 5	314.7	1.51	3	15.1	0.37	3
L14 x R 50	364.9	1.57	3	23.4	0.47	3
L14 x R 55	419.6	1.62	2	18.9	0.40	3
L14 x R 59	186.9	1.23	3	8.7	0.20	3
L14 x R 62	339.0	1.54	1	18.7	0.42	3
L14 x R 66	241.7	1.30	2	13.8	0.37	3
L14 x R 68	272.4	1.41	3	11.1	0.28	3
L14 x R 7	264.0	1.44	2	38.3	0.66	3
L14 x R 70	369.3	1.58	2	14.1	0.35	3
L14 x R 74	296.4	1.48	3	9.4	0.29	3
L14 x R 80	165.8	1.20	3	9.2	0.28	3
L14 x R 82	479.0	1.69	2	12.8	0.28	3
L14 x R 85	278.5	1.44	3	9.1	0.25	3
L14 x R 89	219.7	1.33	3	36.7	0.56	3
L14 x R 91	344.8	1.55	1	37.7	0.50	3
L14 x R 92	116.4	1.06	3	10.5	0.30	3
CxL 14B	195.9	1.28	3	6.9	0.23	3
CxL 3D	322.8	1.49	2	10.1	0.29	3
CxL 41F	464.0	1.66	3	18.8	0.40	3
CxL 42C	295.7	1.47	2	23.2	0.49	3
CxL 43A	284.8	1.46	3	6.4	0.20	3
CxL 48D	267.3	1.41	3	10.4	0.31	3
CxL 5H	321.9	1.44	2	9.3	0.28	3
CxL 6E	278.7	1.44	3	11.7	0.31	3
CxL 7a	358.4	1.56	2	50.5	0.69	3
CxL 8I	322.2	1.51	3	7.3	0.23	3
Rialto	322.7	1.51	3	50.6	0.72	3
Average	**296.1**	**1.44**		**19.8**	**0.40**	
P fung. treatm.		<0.001				
sed (df)		0.033 (2)				
P genotype		<0.001				
sed (df)		0.100 (167)				
P F x G		0.014				
sed (df)		0.144 (167)				

Table 4.6. Area under disease progress curves (AUDPC) in 2014. The average of genotypes within each treatment is reported. The transformation (logarithm base 10 divided by 10) used for the ANOVA is also reported.

Genotype	Untreated AUDPC (% days)	AUDPC (log x/10)	Protected AUDPC (% days)	AUDPC (log x/10)
AxC 117	136.0	0.21	28.0	0.14
AxC 21	104.5	0.20	14.3	0.11
CxL 14B	17.0	0.12	10.5	0.08
CxL 43A	93.0	0.20	16.5	0.09
CxL 5H	52.0	0.17	7.3	0.09
CxL 7A	238.0	0.24	27.3	0.12
L14xR 114	79.8	0.19	36.8	0.16
L14xR 127	123.0	0.21	55.5	0.17
L14xR 133	106.5	0.20	19.0	0.11
L14xR 16	122.5	0.21	25.5	0.12
L14xR 19	346.2	0.25	48.5	0.17
L14xR 20	61.5	0.18	6.3	0.07
L14xR 22	274.8	0.24	68.5	0.18
L14xR 92	57.8	0.18	24.0	0.14
L8xR 10	42.0	0.15	2.0	0.03
L8xR 86	43.3	0.16	15.0	0.12
Average	**118.6**	**0.19**	**25.3**	**0.12**
P fung. treatm.		0.037		
sed (df)		0.015 (2)		
P genotype		<0.001		
sed (df)		0.015 (60)		
P FT x G		0.027		
sed (df)		0.021 (60)		

4.3.4 Presence of other diseases

The 2011-12 season had overall high disease pressure; in addition to STB, Fusarium head blight, eyespot and sharp eyespot were present in the field crop. There were no differences between fungicide treatments for FHB, but there were differences between genotypes ($p<0.001$). The Fusarium head blight severities varied from 18.5 to 67.1% in the protected treatment, and from 23.3% to 68.9% in the untreated. There was no FT x G interaction for FHB. Eyespot severity, expressed as an index from 0-1, did not differ between treatments, but there were differences between genotypes ($p<0.001$). However, unlike the other diseases, both treatments were equally affected by eyespot. There was no FT x G effect. Sharp eyespot, also expressed as an index between 0 - 1, showed no differences between treatments or genotypes. There was a much lower disease incidence of sharp eyespot (29.6% of plots) compared to common eyespot and FHB (87.8% and 100% incidence, respectively). The 2012-13 season had extremely low disease pressure, with virtually no disease present.

In 2013-14, yellow rust was the most prevalent disease after Septoria tritici blotch. There was no difference in yellow rust severity between treatments, but there were differences between lines in flag-leaf severity (<0.001) in all assessments except at GS59 + 26 days. There were also FT x G effects on the flag leaves at GS39 ($p=0.001$), GS59 and GS59 + 14 days ($p<0.001$). There were no significant differences in the lower leaf layers at any assessment. The yellow rust incidence peaked at GS59 at 66.7% and 50% in the untreated and protected plots, respectively.

4.3.5 Green leaf area, green area index, and healthy area duration

As previously mentioned, 2011-12 was a season of high disease pressure and this also affected green leaf area percentage (GLA%) - especially in untreated plots. At GS59, there were differences in GLA% between treatments (86 % protected vs. 59 % untreated) in the third leaf layer ($p=0.028$) but not in the two upper layers. There were significant differences between lines and significant FT x G interactions in all leaf layers at this assessment (Table 4.7). There were differences in GLA% between treatments, lines and G x E interactions in all leaf layers in the assessments at GS59 + 14 days and GS59 + 28 days (Table 4.7). In 2013-14, there was a difference between treatments in flag-leaf GLA% at GS59 ($p=0.041$; 97.5%

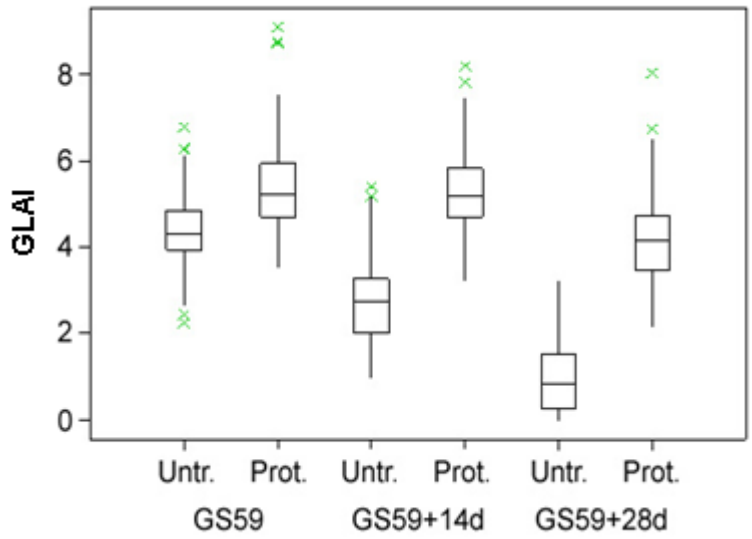
treated vs. 95% untreated) but not in the following two assessments (Table 4.7). There were differences between treatments in the lower leaf layers at all assessments except for the second leaf layer at GS59 (Table 4.7). There were significant differences between lines and significant FT x G interactions in all leaf layers at all assessments (Table 4.7).

The green lamina area index (GLAI) varied between seasons. In 2012, there were differences in GLAI between treatments in all assessments ($p=0.01$; $p=0.005$; $p=0.001$ for GS59; GS59 + 14d and GS59 + 28d, respectively, Fig 4.7a). There were also differences between genotypes and a FT x G interaction at all assessments ($p<0.001$). In 2014, there were differences in GLAI between treatments at GS59 and GS59 + 26 days ($p=0.006$ and $p=0.007$, respectively, Fig 4.7b) and a strong trend for a difference between treatments at GS59 + 14 days ($p=0.062$). Genotypes differed at all assessments ($p<0.001$), but the only significant FT x G interaction ($p = 0.032$) was at GS59 + 26 days. In 2013, there were differences between genotypes at all assessments ($p<0.001$), but there were no differences between treatments.

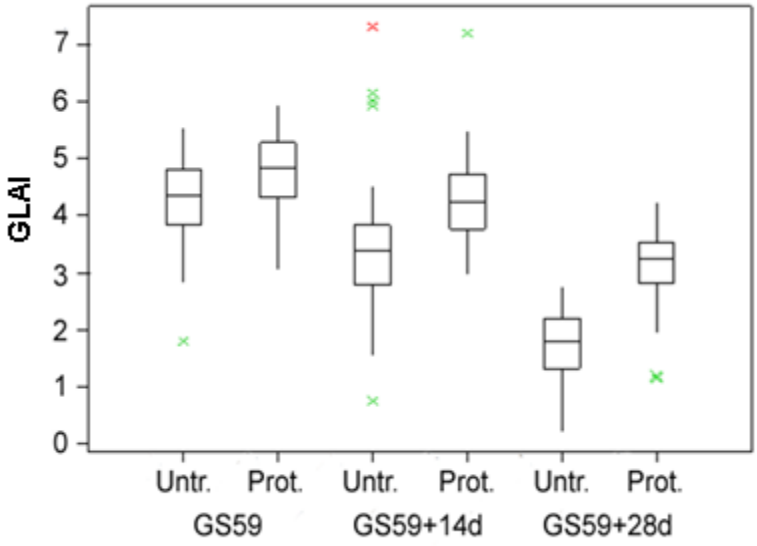
Treatments differed in healthy area duration (HAD) during the grain filling period ($p=0.002$, 83 untreated vs. 190 protected GLAI days) in 2012. The effect of genotypes ($p<0.001$) and FT x G in 2012 ($p<0.001$, Table 4.8) were significant. The HAD values ranged from 43 – 146 in untreated and 142 – 230 GLAI days in protected plots. Due to the loss of green area to drought there were no differences in HAD between treatments in 2013, but there were differences between genotypes in the range 52 – 93 GLAI days ($p<0.001$). There were no differences in HAD between the CIMMYT L14/8-derived and UK-derived genotype groups. There were differences in HAD between treatments ($p=0.002$) and within ($p<0.001$) the CIMMYT L14/8-derived genotypes, with ranges of 43.0 – 123.6 (untreated) and 144.9 – 229.6 GLAI days (protected) and also significant FT x G interactions ($p<0.001$). There were also differences between treatments ($p=0.006$) within the UK genotypes ($p<0.001$) and FT x G interaction ($p=0.028$), although the ranges were smaller at 71.0 – 100.5 and 141.9 – 215.3 GLAI days for untreated and protected lines.

In 2014, there was a significant difference between treatments ($p=0.016$) and lines ($p<0.001$), and a FT x G interaction ($p<0.001$, table 4.9).

Untreated HAD ranged from 43 – 118 and fungicide protected plots ranged 84 – 160 GLAI days.



a)



b)

Fig 4.6. Green lamina area index (GLAI) for the fungicide-protected and untreated treatments in the field experiments on three assessment dates. A) 49 genotypes in 2012 experiment; B) 16 genotypes in 2014 experiment. Outliers, more than 1.5 interquartile ranges from the upper quartile, are displayed in green while far outliers (more than 3 interquartile ranges from the upper quartile) are displayed in red.

Table 4.7. Green flag-leaf area as percentage and expressed as cm^2 per leaf for 2012 and 2014 field seasons.

			Percentage (%)					Area (cm^2)				
Year	**Observation**	**Leaf**	**Avg. untreated**	**Avg. protected**	**P treatment SED (df)**	**P Genotype SED (df)**	**P Trt*Gen SED (df)**	**Avg. untreated**	**Avg. protected**	**P treatment SED (df)**	**P Genotype SED (df)**	**P FT x G SED (df)**
2012	GS59	1	93.9	96.5	**NS** *	**<0.001** 1.16 (192)	**<0.001** 1.64 (192)	36.3	37.7	**NS** *	**<0.001** 2.31 (192)	**NS** *
		2	83.1	93.6	**NS** *	**<0.001** 2.52 (192)	**<0.001** 3.57 (192)	30.8	35.1	**0.017** 0.57 (2)	**<0.001** 1.87 (192)	**NS** *
		3	59.2	85.8	**0.028** 4.55 (2)	**<0.001** 4.47 (192)	**<0.001** 6.32 (192)	17.8	26.1	**0.015** 1.03 (2)	**<0.001** 2.12 (192)	**<0.001** 3.00 (192)
	GS59+14 d	1	81.7	94.5	**0.024** 2.01 (2)	**<0.001** 2.23 (192)	**<0.001** 3.15 (192)	29.7	35.4	**0.002** 0.27 (2)	**<0.001** 2.54 (192)	**0.005** 3.59 (192)
		2	54.4	91.0	**0.025** 5.89 (2)	**<0.001** 4.95 (192)	**<0.001** 7.00 (192)	18.6	32.8	**0.018** 1.94 (2)	**<0.001** 2.43 (192)	**<0.001** 3.43 (192)
		3	20.9	82.0	**0.011** 6.38 (2)	**<0.001** 5.99 (192)	**<0.001** 8.47 (192)	5.5	23.4	**0.008** 1.61 (2)	**<0.001** 2.17 (192)	**<0.001** 3.08 (192)
	GS59+28 d	1	41.4	89.9	**0.016** 6.30 (2)	**<0.001** 6.90 (192)	**<0.001** 9.76 (192)	14.7	33.6	**0.021** 2.81 (2)	**<0.001** 4.11 (192)	**<0.001** 5.81 (192)
		2	11.8	82.2	**<0.001** 0.62 (2)	**<0.001** 5.74 (192)	**<0.001** 8.12 (192)	4.0	28.5	**<0.001** 0.43 (2)	**<0.001** 2.50 (192)	**<0.001** 3.54 (192)
		3	1.3	56.2	**0.017** 7.23 (2)	**<0.001** 7.46 (192)	**<0.001** 10.54 (192)	0.4	15.7	**0.015** 1.92 (2)	**<0.001** 2.31 (192)	**<0.001** 3.27 (192)
2014	GS59	1	95.0	97.5	**0.041** 0.52 (2)	**<0.001** 1.49 (60)	**<0.001** 2.11 (60)	38.0	38.7	**NS** *	**<0.001** 2.31 (60)	**NS** *
		2	92.6	96.8	**NS** *	**<0.001** 1.80 (60)	**<0.001** 2.54 (60)	33.6	35.0	**0.007** 0.12 (2)	**<0.001** 1.53 (60)	**NS** *
		3	79.5	92.7	**0.028** 2.23 (2)	**<0.001** 4.69 (60)	**0.001** 6.63 (60)	21.8	27.3	**0.018** 0.73 (2)	**NS** *	**NS** *
	GS59+14 d	1	90.9	96.2	**NS** *	**0.001** 2.69 (60)	**0.015** 3.80 (2)	36.9	36.1	**NS** *	**NS** *	**NS** *
		2	81.0	94.2	**0.037** 2.60 (2)	**<0.001** 3.96 (60)	**<0.001** 5.61 (60)	28.5	35.7	**NS** *	**NS** *	**NS** *
		3	55.0	85.1	**0.019** 4.26 (2)	**<0.001** 6.11 (60)	**<0.001** 8.63 (60)	13.5	23.2	**0.005** 0.68 (2)	**<0.001** 1.82 (58)	**0.002** 2.57 (58)
	GS59+28 d	1	74.3	89.9	**NS** *	**<0.001** 4.49 (60)	**<0.001** 6.35 (60)	25.1	32.3	**0.018** 0.97 (2)	**<0.001** 2.29 (60)	**<0.001** 3.23 (60)
		2	44.4	83.7	**0.024** 6.18 (2)	**<0.001** 6.54 (60)	**<0.001** 9.23 (60)	13.3	27.6	**0.017** 1.88 (2)	**<0.001** 2.26 (60)	**<0.001** 3.20 (60)
		3	5.8	54.6	**0.009** 4.70 (2)	**<0.001** 6.29 (60)	**<0.001** 8.89 (60)	1.27	13.2	**0.005** 0.86 (2)	**<0.001** 1.67 (60)	**<0.001** 2.36 (60)

Table 4.8. Healthy area duration (HAD) from GS59 to physiological maturity during the grain filling period in 2012.

Genotype	Untreated (GLAI days)	Protected (GLAI days)
L14 x R 05	93.0	221.9
L14 x R 07	78.0	177.2
L14 x R 10	43.0	158.9
L14 x R 104	51.3	167.1
L14 x R 107	69.5	169.1
L14 x R 112	79.7	159.1
L14 x R 114	116.9	166.0
L14 x R 119	94.4	216.4
L14 x R 121	126.8	193.2
L14 x R 123	145.9	279.4
L14 x R 127	90.2	153.0
L14 x R 129	88.6	162.2
L14 x R 131	73.1	165.8
L14 x R 133	73.9	195.7
L14 x R 136	66.4	203.0
L14 x R 138	79.6	229.6
L14 x R 15	88.7	224.9
L14 x R 16	65.8	199.1
L14 x R 19	67.6	182.8
L14 x R 20	88.6	181.1
L14 x R 22	46.7	144.9
L14 x R 23	64.6	204.6
L14 x R 35	84.1	196.8
L14 x R 43	68.1	166.7
L14 x R 50	83.3	226.2
L14 x R 55	67.4	192.8
L14 x R 59	99.4	185.2
L14 x R 62	57.7	188.4
L14 x R 66	71.8	175.6
L14 x R 68	123.6	228.0
L14 x R 70	80.6	225.2
L14 x R 74	82.1	201.6
L14 x R 80	104.9	228.5
L14 x R 82	78.6	224.3
L14 x R 85	75.1	188.3
L14 x R 89	94.2	213.1
L14 x R 91	52.2	122.7
L14 x R 92	123.2	184.2
CxL 14B	123.3	197.1
CxL 3D	76.5	166.4
CxL 41F	71.0	173.2
CxL 42C	77.2	170.0
CxL 43A	85.8	199.1
CxL 48D	76.1	166.6
CxL 5H	100.5	215.3
CxL 6E	76.0	188.7
CxL 7a	62.9	141.9
CxL 8I	76.0	201.9
Rialto	78.9	201.8
Grand Total	**82.5**	**190.3**
P treatment	0.002	
SED (df)	5.11 (2)	
P genotype	<0.001	
SED (df)	10.02 (192)	
P FT x G	<0.001	
SED (df)	14.17 (192)	

Table 4.9. Healthy area duration (HAD) from GS59 to physiological maturity during the grain filling period in 2014.

Genotype	Untreated (GLAI days)	Protected (GLAI days)
AxC 117	102.0	123.2
AxC 21	104.3	145.7
CxL 14B	114.4	147.7
CxL 43A	110.7	159.9
CxL 5H	101.2	155.5
CxL 7A	62.7	122.2
L14xR 114	110.5	134.4
L14xR 127	69.4	84.1
L14xR 133	86.6	130.7
L14xR 16	82.4	133.9
L14xR 19	43.4	105.6
L14xR 20	96.8	147.6
L14xR 22	49.6	86.1
L14xR 92	107.7	130.0
L8xR 10	117.8	135.3
L8xR 86	93.0	109.7
Grand Total	**90.8**	**128.2**
P treatment	0.016	
SED (df)	5.04 (2)	
P genotype	<0.001	
SED (df)	7.15 (60)	
P FT x G	<0.001	
SED (df)	20.21 (60)	

4.3.6 Grain yield and yield components

Grain yield differed between genotypes in all three field experiments, and between fungicide treatments in 2012 and 2014, but not in 2013.

In 2012, yield differed between treatments ($p=0.017$), genotypes ($p<0.001$), and there was a significant G x FT interaction ($p<0.001$; Table 4.10). The untreated plots yielded 24.10% less (5.73 t ha^{-1}) than the protected (7.63 t ha^{-1}) and ranged from 3.78 - 7.79 t ha^{-1} compared to 4.50 - 10.34 t ha^{-1} in the protected plots (Fig 4.7). In 2013 mean yield was higher than 2012; 9.54 t ha^{-1}, but there was a smaller overall range (7.55 to 11.51 t ha^{-1}) and no significant difference between treatments. There were differences between genotypes ($p<0.001$), but there was no FT x G interaction. In 2014, grain yield was reduced by 19.0% from 7.88 t ha^{-1} in the protected treatment to 6.38 t ha^{-1} in the untreated ($p=0.046$). There were also differences between genotypes ($p<0.001$) and a FT x G interaction ($p<0.001$; Table 4.11). The genotype ranges were the smallest of the three field experiments; between 6.44 to 9.43 t ha^{-1} in fungicide protected plots, and 5.01 to 8.07 t ha^{-1} in untreated plots (Fig 4.7).

In 2012, the parent Rialto was one of the highest yielding genotypes under fungicide protection at 10.34 t ha^{-1}, but only yielded 6.57 t ha^{-1} when untreated. The L14 x Rialto DH lines yielded an average 7.26 t ha^{-1} (genotypic range 4.50 – 9.14 t ha^{-1}) when fungicide protected and 5.61 t ha^{-1} (3.78 – 7.79 t ha^{-1}) when untreated. The Cadenza x Lynx DH reference lines had a slightly higher fungicide-protected yield at 8.73 t ha^{-1} (6.39 – 10.34 t ha^{-1}) and about 0.5 t ha^{-1} higher untreated yield at 6.11 t ha^{-1} (3.90 – 7.39 t ha^{-1}). When the two DH populations were analysed separately, the L14 x Rialto DH lines differed in yield between treatments ($p=0.021$) and genotypes ($p<0.001$) and there was a FT x G effect ($p<0.001$). The Cadenza x Lynx lines on the other hand displayed yield differences between treatments and genotypes ($p=0.011$ and $p=0.009$, respectively), but no FT x G interaction.

In 2013, there were a larger number of high yielding UK varieties with an average yield of 10.79 t ha^{-1}, with a genotypic range of 9.74 – 11.51 t ha^{-1}. When this group was analysed separately there were no differences between treatments or genotypes, and no interactions. The Cadenza x Lynx DH reference lines yielded on average 9.64 t ha^{-1} (9.10 – 10.69 t ha^{-1}) and displayed no differences between treatments. There was a trend for differences between genotypes ($p=0.055$), and no interaction between

treatment and genotype. The L8 x Rialto DH lines yielded an average 8.63 t ha^{-1}, ranging from 7.55 to 9.86 t ha^{-1}. There was a difference between genotypes in this group (p=0.005), but not between treatments and no interaction.

In 2014, there was no difference in untreated grain yield between the CIMMYT L14/8 x Rialto DH lines (L14 x Rialto and L8 x Rialto) and the UK DH lines (Lynx x Cadenza and Avalon x Cadenza), but the UK DH lines had a higher fungicide-protected yield (8.4 t ha^{-1} vs. 7.6 t ha^{-1}; p<0.001). Disease reduced the average yield of the UK lines from 8.4 to 6.3 t ha^{-1} (p=0.056), and from 7.6 to 6.5 t ha^{-1} in the CIMMYT L14/8 x Rialto DH lines (p=0.037). When untreated, the CIMMYT L14/8 x Rialto DH lines ranged from 5.1 t ha^{-1} to 7.3 t ha^{-1} and the UK-derived lines had a slightly larger range between 5.0 to 8.1 t ha^{-1}. In the fungicide protected treatment, the CIMMYT L14/8 x Rialto DH lines varied between 6.4 and 8.8 t ha^{-1} and the UK DH lines from 7.5 to 9.4 t ha^{-1}.

Grains per m^2 and thousand grain weight (TGW) also differed between lines and treatments. In 2012, grains m^{-2} in untreated plots was 15,501 compared to 18,713 in protected plots (p=0.017). There were also differences between genotypes and a FT x G interaction (p<0.001). Genotypes ranged from 9,070 - 19,567 grains m^{-2} in untreated plots and from 9,226 - 25,087 grains m^{-2} in fungicide-protected plots. In 2013, there was no difference between treatments (20,515 untreated vs. 20,197 grains m^{-2} treated). There were differences between genotypes in the range 15,710 – 25,278 (p<0.001). In 2014, grains m^{-2} was reduced from 20,345 in protected plots to 17,566 grains m^{-2} in untreated plots (p=0.032). There was a difference between genotypes (p<0.001) and an FT x G interaction (p<0.001). Genotypes in untreated plots ranged from 13,828 to 20,463 grains m^{-2}, and in the protected plots from 17,517 and 22,960 grains m^{-2}.

There were no differences in ears per m^2 between fungicide treatments, and no FT x G interaction in 2012, but there were differences between genotypes (p=0.006). Ears m^{-2} overall was 508 in 2012, with a genotype range from 425 - 676. Ears m^{-2} was considerably lower in 2013 at 368 ears per m^2. There were no differences between treatments and no FT x G interaction, but genotypes ranged from 290 to 428 ears m^{-2}. In 2014, as in previous years, there were no significant differences between fungicide treatments, and no FT x G interaction. There were differences between genotypes in the range of 369 - 485 ears per m^2 (p=0.007).

Grains per ear increased from 29.9 in untreated to 39.6 in protected pots in 2012 ($p=0.029$). Genotypes ranged from 14.6 – 43.7 for the untreated and 15.6 – 63.8 grain ear^{-1} in the protected plots ($p<0.001$). In addition there was a strong FT x G interaction ($p<0.001$). There were no significant differences in grains ear^{-1} between treatments in 2013 (57.1 in untreated vs. 56.0 in protected), and no FT x G interaction. The genotypes differed in the range 47.4 – 65.7 grains ear^{-1}. Similarly, there was no significant difference between treatments in 2014; the mean value for the untreated plots was 44.3 and the protected plots 48.5 grains per ear. There was no FT x G interaction, but there were differences between genotypes in the range of 38.8 – 56.0 grains ear^{-1} ($p<0.001$).

In 2012, the mean untreated TGW was 36.2 g and the fungicide-protected mean 40.2 g ($p<0.001$). There were also differences between genotypes ($p<0.001$) and a FT x G interaction ($p=0.003$). In the untreated plots genotypes ranged from 26.7 to 45.7 g and from 30.4 to 48.1 g in the fungicide-protected plots. In 2013, there was no significant fungicide effect (untreated 46.9 g and protected 47.2 g); genotypes differed in the range 41.0 – 52.2 g ($p<0.001$), but there was no FT x G interaction. There was no significant difference in TGW between treatments in 2014 (untreated mean TGW 43.1 g and protected TGW 46.2 g). There were, however, differences between genotypes ($p<0.001$) and a FT x G interaction ($p<0.001$). The genotypes ranged between 31.9 g and 50.5 g in untreated plots, and between 39.7 g and 50.9 g in protected plots.

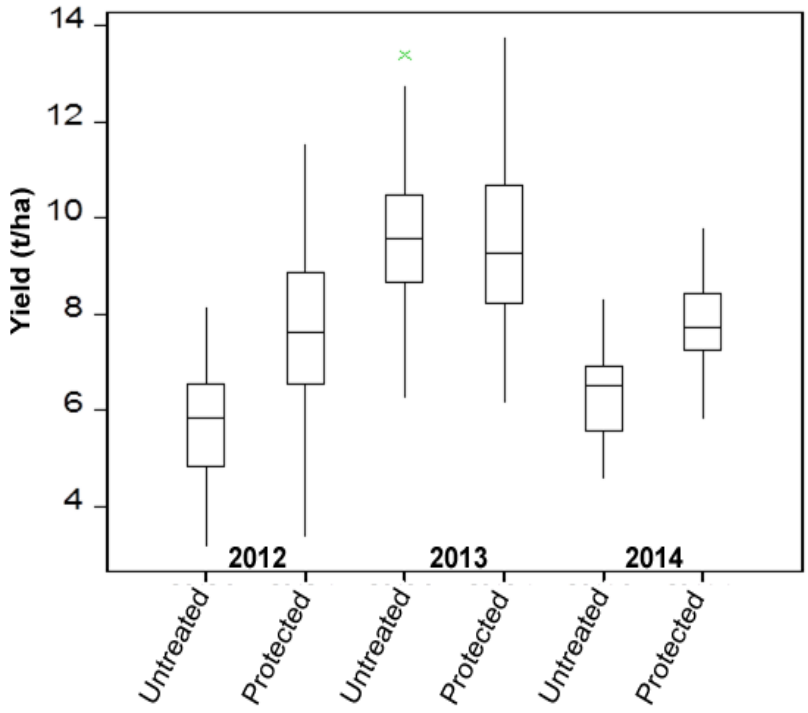


Fig 4.7. Grain yield (t ha^{-1}) at 85% DM for 49 genotypes in 2012, 40 genotypes in 2013 and 16 genotypes in 2014, in untreated and protected plots. Outliers (more than 1.5 interquartile ranges from the upper quartile) are shown in green.

Table 4.10. Grain yield (85% DM) and yield components in the 2011/12 field experiment.

	Untreated			Protected		
Genotype	Yield (t ha^{-1})	TGW (g)	Grains m^{-2}	Yield (t ha^{-1})	TGW (g)	Grains m^{-2}
L14 x R 10	4.0	28.9	12789.7	7.1	34.0	20260.5
L14 x R 104	4.6	34.3	14023.3	8.5	44.9	25170.0
L14 x R 107	5.8	31.1	16191.0	7.8	32.8	16054.7
L14 x R 112	3.8	40.4	10715.4	4.8	43.0	12639.9
L14 x R 114	6.8	30.8	17114.4	7.6	32.1	17036.2
L14 x R 119	5.5	31.8	18268.0	7.5	32.7	22767.4
L14 x R 121	7.2	43.0	22007.1	7.9	42.6	22942.5
L14 x R 123	7.3	35.1	18065.7	9.1	41.4	21257.7
L14 x R 127	7.8	40.0	23146.3	7.8	38.7	19877.3
L14 x R 129	5.3	41.6	13108.1	7.3	46.3	16161.2
L14 x R 131	6.6	33.2	15747.4	6.4	41.9	13765.3
L14 x R 133	6.4	36.4	20275.2	9.1	46.4	21574.1
L14 x R 136	4.4	29.7	11589.8	8.4	35.9	17223.4
L14 x R 138	5.2	37.2	16327.4	6.9	38.5	19094.1
L14 x R 15	6.1	42.1	12165.7	7.4	39.8	19050.4
L14 x R 16	5.0	30.2	16427.6	8.1	35.4	21928.1
L14 x R 19	5.9	39.5	14688.1	6.7	38.6	18186.8
L14 x R 20	5.3	33.5	15211.3	5.5	33.2	17509.6
L14 x R 22	5.1	30.1	17043.5	9.8	35.5	27665.9
L14 x R 23	6.0	31.9	18093.4	7.6	32.4	25041.8
L14 x R 35	6.1	32.1	18026.3	8.0	32.2	22637.5
L14 x R 43	5.1	45.5	10504.5	6.1	44.7	13643.5
L14 x R 5	4.4	26.0	16959.6	7.1	28.1	25322.4
L14 x R 50	4.2	41.1	9190.0	6.5	44.1	12322.7
L14 x R 55	5.3	34.8	16224.0	7.8	42.6	18081.0
L14 x R 59	4.0	45.5	7894.5	4.5	43.3	10483.6
L14 x R 62	4.9	31.8	13843.4	7.4	37.6	17619.4
L14 x R 66	5.6	31.8	17285.7	7.6	33.0	22498.2
L14 x R 68	6.6	35.5	18328.1	6.2	34.1	18812.2
L14 x R 7	5.0	33.4	14106.8	6.4	35.5	18004.2
L14 x R 70	6.1	32.4	17871.9	7.5	30.7	26686.8
L14 x R 74	5.2	42.9	11765.6	6.1	41.7	14810.8
L14 x R 80	4.6	41.5	11699.6	4.7	45.7	11196.9
L14 x R 82	6.4	33.2	15401.9	8.4	34.9	17823.6
L14 x R 85	7.1	38.5	20135.7	7.0	36.0	21784.5
L14 x R 89	5.9	35.5	15554.3	8.9	40.0	23365.0
L14 x R 91	5.2	36.0	14263.9	6.5	41.2	16253.7
L14 x R 92	7.1	32.6	19685.1	8.5	33.2	17962.5
CxL 14B	7.4	37.3	20253.2	9.5	40.0	23327.5
CxL 3D	5.6	31.2	15848.4	10.3	41.5	22484.3
CxL 41F	6.1	37.7	20202.7	7.6	42.9	20973.2
CxL 42C	6.0	34.7	15284.9	7.7	35.1	19541.6
CxL 43A	6.6	36.5	19571.8	9.6	38.5	22448.0
CxL 48D	7.3	35.0	19217.8	9.0	40.1	22130.9
CxL 5H	7.3	35.1	27252.7	9.0	41.4	27865.3
CxL 6E	4.2	27.1	10856.2	9.0	34.1	21344.0
CxL 7a	3.9	26.5	9954.2	6.5	31.1	17191.4
CxL 8I	6.6	35.2	19061.8	9.2	38.6	25392.4
Rialto	6.6	32.3	20453.6	10.3	40.0	24080.0
Average	5.7	35.1	16101.1	7.7	38.2	19884.6
P treatment	0.018	<0.001	0.017			
SED (df)	0.26 (2)	0.025 (2)	419.7 (2)			
P genotype	<0.001	<0.001	<0.001			
SED (df)	0.54 (187)	1.66 (191)	1283.8 (186)			
P FT x G	<0.001	0.003	<0.001			
SED (df)	0.76 (187)	2.35 (191)	1815.5 (186)			

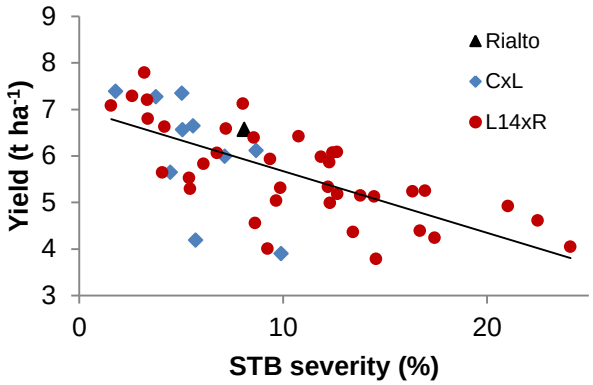
Table 4.11. Grain yield (85% DM) and yield components in the 2013/14 field experiment

Genotype	Untreated Yield (t ha^{-1})	TGW (g)	Grains m^{-2}	Protected Yield (t ha^{-1})	TGW (g)	Grains m^{-2}
AxC 117	5.5	37.6	17102.7	8.3	43.2	22711.4
AxC 21	5.3	40.0	15644.0	7.5	44.7	19843.4
CxL 14B	8.1	47.1	20354.4	9.0	47.5	22595.1
CxL 43A	5.8	45.7	15130.0	7.5	48.6	18333.4
CxL 5H	7.9	46.4	20162.0	9.4	49.2	22960.5
CxL 7A	5.0	31.9	18428.6	8.5	39.7	25325.1
L14xR 114	6.8	43.7	18251.2	7.6	43.7	20547.2
L14xR 127	6.0	49.4	14377.3	7.5	50.4	17516.9
L14xR 133	7.2	50.5	17045.2	7.7	50.9	18011.8
L14xR 16	6.8	44.0	18277.5	8.8	49.7	21169.7
L14xR 19	5.1	43.2	13828.3	7.2	49.9	17220.0
L14xR 20	6.6	44.7	17390.4	7.7	49.5	18344.9
L14xR 22	5.4	39.1	16218.7	7.6	43.3	20762.4
L14xR 92	7.3	42.1	20463.0	7.9	43.1	21786.1
L8xR 10	6.6	41.0	19250.5	6.4	41.7	18348.3
L8xR 86	6.9	42.5	19139.1	7.4	43.9	20049.2
Average	**6.4**	**43.1**	**17566.4**	**7.9**	**46.2**	**20345.3**
P treatment	0.046	0.125	0.032			
SED (df)	0.33 (2)	1.23 (2)	510.11 (2)			
P genotype	<0.001	<0.001	<0.001			
SED (df)	0.25 (60)	1.01 (60)	672.42 (60)			
P FT x G	<0.001	<0.001	<0.001			
SED (df)	0.355 (60)	1.43 (60)	950.95 (60)			

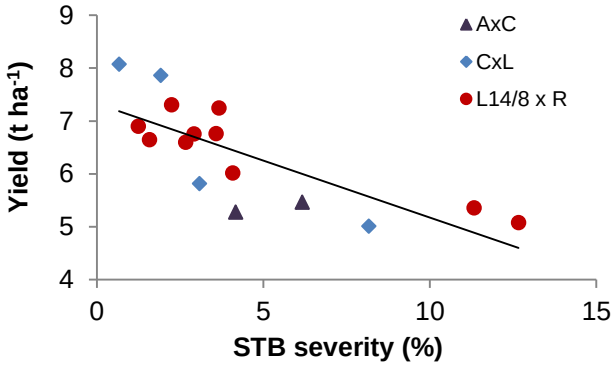
4.3.7. Relationship between disease and grain yield

Genetic variation in Septoria tritici blotch disease severity had effects on untreated yields in both 2012 and 2014. There was a linear relationship amongst genotypes between untreated yield and flag-leaf STB severity at GS59 and GS59+14d in 2012. At GS59, the relationship was significant but weak ($p<0.001$) accounting for 10.6 % of the yield variation. The second leaf STB severity also had an effect ($p<0.001$), accounting for 18.2% of the yield variation. The strongest relationship was found at GS59 + 14 days, where the linear relationship between flag-leaf STB severity and grain yield amongst genotypes accounted for 34.6% of the variation in untreated yield ($p<0.001$). There was no relationship between flag-leaf disease severity at GS59 + 28 days and grain yield, probably due to poor disease representation at this assessment as discussed earlier.

In 2014, there were also significant relationships between STB severity and untreated yield. At GS59, there were linear relationships between STB severity of leaf 2 ($p=0.002$) and leaf 3 ($p<0.001$) and untreated yield, accounting for 16.6% and 29.6% of yield variation, respectively. At GS59 + 14 days there were significant relationships between STB severity of the top three leaf layers and untreated yield. The strongest relationship was once again found for the flag-leaf STB severity ($p<0.001$), which accounted for 41.4% of the yield variation. The STB severity of leaf 2 and leaf 3 ($p<0.001$ and $p=0.006$, respectively) accounted for 27.2% and 13.9% of variation in yield, respectively. There were linear relationships between STB severity on each of the flag leaf and the third leaf layer and untreated yield at GS59+26 days ($p<0.001$ and $p=0.026$, respectively). The STB symptoms present on the flag leaf accounted for 36.8% of variation in yield amongst genotypes, while those on the third leaf layer accounted for 20.5%.



a)



b)

Fig 4.8 Linear regression of grain yield (85% DM) on Septoria disease for 49 genotypes in 2012 and 16 genotypes in 2014. Shown are the significant ($p<0.001$) relationships between flag-leaf disease at GS59 + 14 days and untreated yield in a) 2012 field season and b) 2014 field season.

In order to determine the disease effect on yield integrated over the entire season, the relationships between AUDPC and grain yield was determined. In 2012, there was a weak relationship between untreated AUDPC and untreated grain yield ($p=0.002$, $r^2=0.071$), but no relationship between protected AUDPC and protected grain yield. In 2014, there was a much stronger relationship between untreated AUDPC and untreated grain yield ($p<0.001$) accounting for 41.2% of the variation (Fig 4.9). There was no relationship between the fungicide protected AUDPC and grain yield.

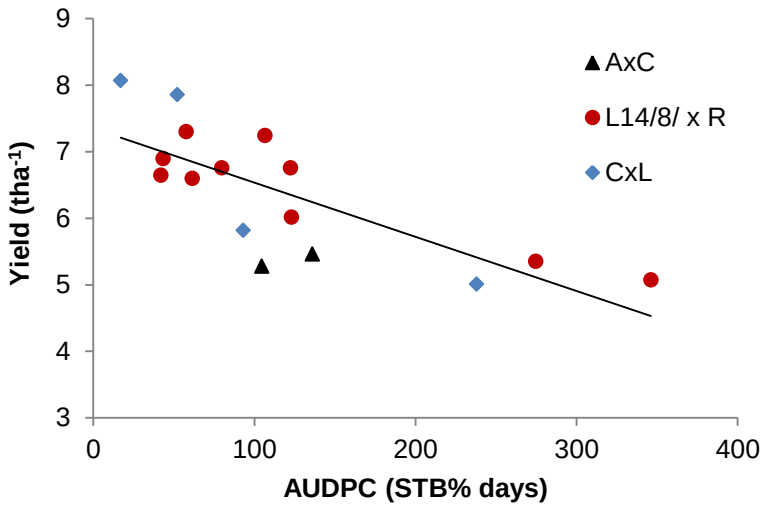


Fig 4.9. The relationship between untreated flag-leaf AUDPC and untreated grain yield in 2014 ($p<0.001$, $r^2=0.412$)

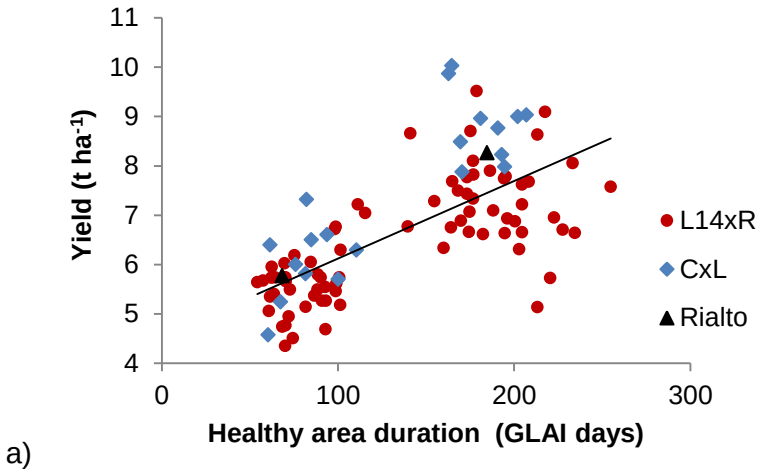
Effects of genotype on yield in the treatments can be more easily interpreted by considering the components of yield; grains m^{-2} and TGW. Regression analysis indicated a negative linear relationship ($p<0.001$) amongst genotypes between untreated grains m^{-2} and flag leaf STB disease severity at GS59 + 14 days in 2012, accounting for 22.2% of yield variation. In contrast, there was only a weak, but significant, relationship between flag leaf STB at the same assessment and untreated TGW ($p<0.001$, $r^2=0.034$). There was also a negative linear relationship between flag-leaf STB and grains ear^{-1} (accounting for 18.5 % variation, $p<0.001$). In 2014, there were linear relationships between each of untreated grains m^{-2} and TGW and flag-leaf STB disease severity at GS59 + 14 days. However, as in 2012, the relationship between TGW and STB was weaker ($p=0.007$, $r^2=0.142$) than that

between grains m^{-2} and STB severity ($p<0.001$, $r^2=0.221$). There was only a weak relationship between grains per ear and flag leaf STB in this experiment ($p=0.047$, $r^2=0.031$).

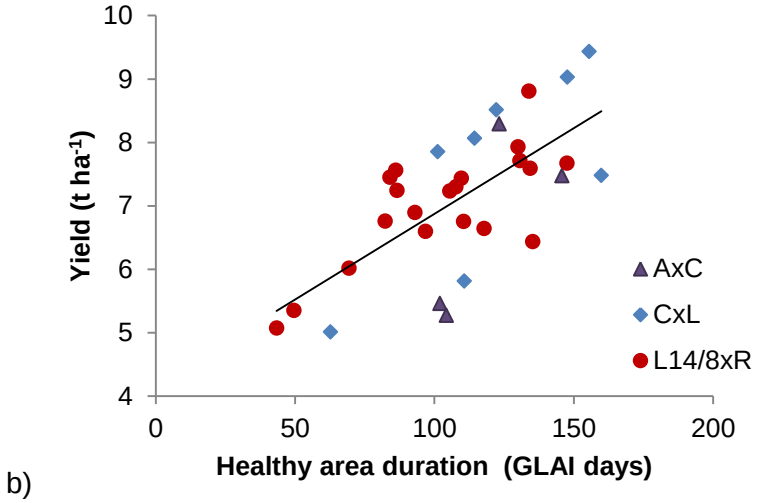
There were other diseases present in the field experiments in addition to Septoria tritici blotch. There was high Fusarium head blight severity in the 2012 field season. However, as mentioned previously, there was no significant difference in FHB severity between treatments and no FT x G interaction. There was a linear relationship between FHB and untreated yield ($p<0.001$) but it was very weak, accounting for only 3.5% variation amongst genotypes. In addition, there was eyespot and sharp eyespot present in 2012. Eyespot was also shown to have a linear ($p<0.001$), but very weak relationship with untreated yield ($r^2=0.044$), while sharp eyespot had no relationship with grain yield. A multiple linear regression analysis indicated that FHB and eyespot accounted for 10.2% of grain yield variation ($p<0.001$), while sharp eyespot did not affect yield.

The main disease in 2014, apart from STB, was yellow rust. There was a linear, but relatively weak, relationship between untreated yield and yellow rust on the flag leaf at GS59 ($p<0.001$, $r^2=0.121$). There was also a linear relationship between yellow rust and untreated yield at the flag leaf level at GS59 + 14 days ($p=0.018$, $r^2=0.092$); however, this was due to very high yellow rust severities in two of the plots; when these were removed there was no relationship.

Healthy area duration during grain filling also had an association amongst genotypes with yield in 2012 ($p<0.001$) and accounted for 30.3% of the yield variation (Fig 4.10). A similar, but stronger relationship amongst genotypes between HAD and yield was seen in 2014 ($p<0.001$), where HAD accounted for 37.2% of the yield variation (Fig 4.10). Despite the correlation between HAD and yield, the relationships between HAD and TGW in 2012 ($p<0.001$, $r^2=0.071$) and 2014 ($p=0.001$, $r^2=0.096$) were relatively weak. Stronger associations were observed between HAD and grains m^{-2} (p $p=0.001$, $r^2=0.16.7$ and $p<0.001$, $r^2=0.243$, respectively).



a)



b)

Fig 4.10. The linear relationships of grain yield (85% DM) on healthy area duration for a) 49 genotypes in the 2012 experiment ($p<0.001$; $r^2=0.303$) and b) 16 genotypes in the 2014 experiment ($p<0.001$; $r^2=0.372$). The genotype averages for each fungicide treatment are included in the graphs.

4.3.8. Disease tolerance

Disease tolerance for a given cultivar is quantified as the linear slope between grain yield per unit area and healthy area duration. In 2012, there were large differences between treatments and genotypes in yield and HAD, and significant differences in tolerance. Similarly, genotypes in 2014 showed significant differences in tolerance. In 2013, however, yield and HAD did not differ significantly between treatments so disease tolerance linear slopes between grain yield per unit area and healthy area duration were not significant and tolerance could not be calculated. Therefore, disease tolerance in 2013 will not be analysed further in this chapter or following chapters.

In 2012, there was a considerable range in disease tolerance (4.4 standard deviations) between the most and least tolerant genotype ($p<0.001$, Table 4.12). There was also a large spread in tolerance in 2014 (3.8 standard deviations) amongst genotypes between highest and lowest tolerance ($p<0.001$, Table 4.13). Forty nine genotypes were screened in 2012, of which 22 had disease intolerance values lower than the 95% confidence interval of the mean, while 17 had values larger than the confidence interval (i.e. significantly more intolerant (Table 4.12)). In 2014, 16 genotypes were screened for tolerance; four of which were more tolerant than the 95% confidence interval, and three of which were more intolerant (Table 4.13).

In general, the L14 x Rialto DH population displayed a wider range of disease tolerance than the Cadenza x Lynx DH reference lines. None of the reference lines displayed a significant disease tolerance in 2012 or 2014 (Table 4.12 -13). In 2014, one of the L8 x Rialto lines (L8 x R10) displayed significant tolerance and the other was in the middle group, that is, neither tolerant nor intolerant. Neither of the Avalon x Cadenza DH lines displayed significant disease tolerance in 2014; however, one of them showed significant intolerance (AxC 117, Table 4.13).

Table 4.12. Disease tolerance slopes (grain yield HAD^{-1}) of genotypes in 2012. Listed below are also the standard error and the p values of the relationship between HAD and yield. The 95% confidence interval from the mean is indicated by the two lines.

Genotype	Slope ($t\ ha^{-1}\ HAD^{-1}$)	SE	P value
L14 x R 85	-0.001	0.004	0.709
L14 x R 131	0.000	0.009	0.970
L14 x R 20	0.000	0.003	0.897
L14 x R 80	0.001	0.003	0.789
L14 x R 19	0.004	0.004	0.367
L14 x R 68	0.004	0.004	0.357
L14 x R 121	0.005	0.005	0.433
L14 x R 59	0.005	0.004	0.233
L14 x R 74	0.009	0.004	0.079
L14 x R 43	0.009	0.003	0.056
L14 x R 70	0.009	0.004	0.066
L14 x R 138	0.010	0.003	0.03
L14 x R 15	0.010	0.001	0.002
L14 x R 114	0.010	0.008	0.253
L14 x R 89	0.011	0.014	0.501
L14 x R 123	0.011	0.006	0.172
L14 x R 23	0.011	0.002	0.005
L14 x R 127	0.012	0.013	0.427
L14 x R 92	0.012	0.011	0.315
L14 x R 112	0.013	0.003	0.008
L14 x R 82	0.014	0.002	0.003
L14 x R 50	0.014	0.004	0.027
CxL 48D	0.015	0.007	0.082
L14 x R 119	0.016	0.004	0.017
L14 x R 107	0.016	0.006	0.061
L14 x R 62	0.016	0.007	0.121
L14 x R 66	0.017	0.008	0.106
L14 x R 55	0.018	0.010	0.154
L14 x R 35	0.019	0.005	0.014
CxL 8I	0.019	0.005	0.015
CxL 14B	0.020	0.009	0.093
L14 x R 5	0.020	0.003	0.004
L14 x R 16	0.021	0.005	0.017
L14 x R 7	0.021	0.005	0.023
L14 x R 133	0.021	0.004	0.007
CxL 42C	0.022	0.018	0.312
CxL 5H	0.024	0.006	0.029
L14 x R 129	0.024	0.009	0.086
CxL 43A	0.025	0.004	0.002
L14 x R 10	0.026	0.002	<.001
L14 x R 136	0.028	0.004	0.003
L14 x R 104	0.029	0.005	0.005
Rialto	0.029	0.007	0.013
L14 x R 91	0.031	0.008	0.03
CxL 41F	0.032	0.007	0.017
CxL 7a	0.038	0.012	0.048
CxL 6E	0.040	0.011	0.022
CxL 3D	0.045	0.010	0.012
L14 x R 22	0.050	0.006	<.001

Table 4.13. Disease tolerance slopes (grain yield HAD^{-1}) for 14 genotypes in 2014, the standard error and the p values of the relationship between yield and HAD. The 95% confidence interval from the mean is indicated by the two lines.

Genotype	Slope ($t\ ha^{-1}HAD^{-1}$)	SE	P value
L8xR 10	0.000	0.008	0.994
L14xR 133	0.010	0.010	0.359
L14xR 114	0.015	0.018	0.463
L14xR 20	0.021	0.004	0.006
L8xR 86	0.023	0.039	0.596
CxL 14B	0.024	0.012	0.118
AxC 21	0.024	0.017	0.225
CxL 5H	0.028	0.005	0.006
L14xR 92	0.030	0.017	0.147
L14xR 19	0.033	0.004	0.001
L14xR 127	0.034	0.025	0.248
CxL 43A	0.034	0.009	0.020
L14xR 16	0.037	0.008	0.008
AxC 117	0.053	0.036	0.219
CxL 7A	0.054	0.009	0.004
L14xR 22	0.060	0.009	0.003

There was a linear relationship between fungicide-treated yield and disease tolerance slope (i.e. intolerance) amongst genotypes accounting for 20.3% of the variation in 2012 ($p<0.001$, fig 4.11), but no relationship with untreated yield. In 2014, there was no relationship between disease tolerance and untreated or fungicide-treated yield.

The disease severities of the genotypes were analysed in order to determine the influence of disease resistance on the tolerance scores. There was no relationship between disease severity (GS59 + 14d) and tolerance in 2012 or 2014, and there was no relationship between crop height and disease tolerance in the experiments.

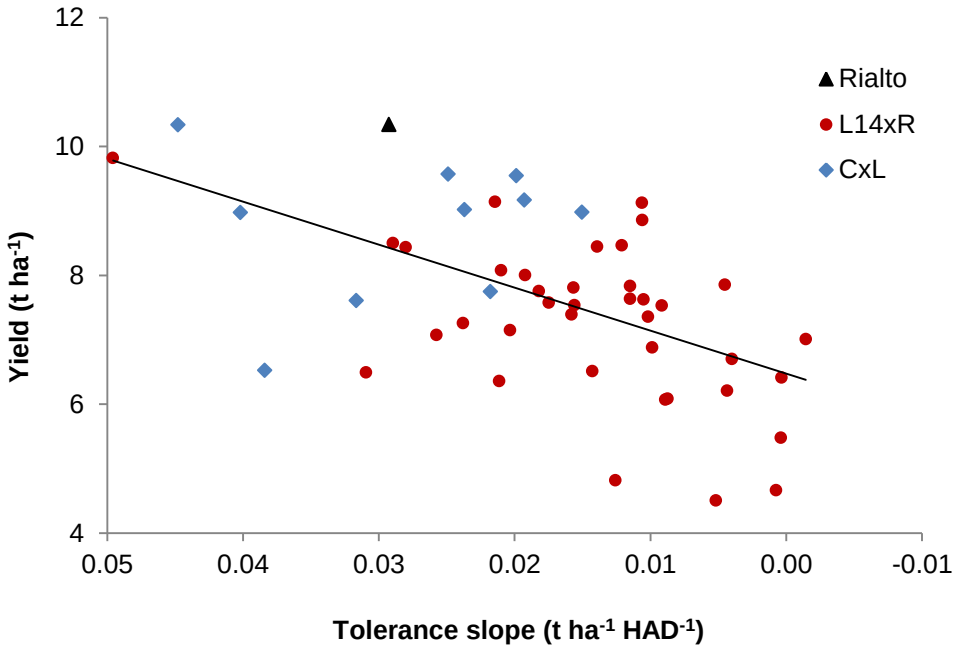


Fig 4.11. The linear relationship between protected yield (85% DM) and disease intolerance for 49 genotypes in the 2012 field experiment ($p<0.001$, $r^2=0.203$).

4.3.9. Consistency of disease tolerance and associated traits between years

Several genotypes were used in more than one field season. The Cadenza x Lynx DH lines were used as reference lines to normalise site and season variation. A number of the Line 14 x Rialto lines were also included in both experiments as they had displayed high or low tolerance in 2012. In total, 12 genotypes were screened in both 2012 and 2014, which allowed the stability of disease tolerance and associated traits to be determined.

For these 12 lines overall the protected yield in 2012 was higher (8.2 t ha^{-1}) than in 2014 (8.0 t ha^{-1}), but not significantly so. Mean untreated yield was 6.2 t ha^{-1} in 2012 and 6.5 t ha^{-1} in 2014 ($p=0.096$). There were year x genotype interactions for both untreated ($p<0.001$) and protected ($p=0.006$) grain yield. There was a linear relationship amongst genotypes between the protected yields in 2012 and 2014 ($p=0.033$, $r^2=0.111$, Fig 4.12), and a slightly stronger relationship between the untreated yields ($p=0.005$, $r^2=0.183$, fig 4.12). There was a strong relationship in untreated yields in 2012 and 2014 amongst the Cadenza x Lynx DH lines ($p<0.001$, $r^2=0.623$). The L14 x Rialto

DH lines had a very large residual variation and could not be accurately analysed separately (Fig 4.12).

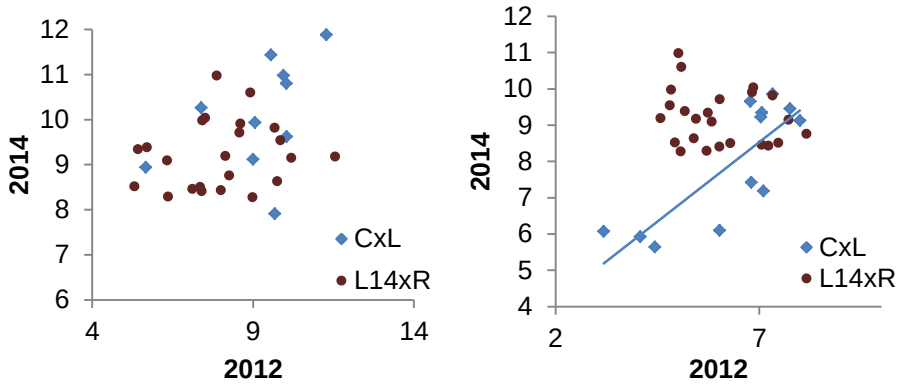


Fig 4.12. The relationships between the 2012 and 2014 field seasons in protected yield and untreated yield. A) Protected yield with the Cadenza x Lynx population (r^2:0.11) and L14 x Rialto population (r^2:n/a) shown separately. B) Untreated yield with the Cadenza x Lynx population (r^2:0.62) and L14 x Rialto population (r^2:n/a) shown separately.

There was no significant association found in percentage yield loss across all genotypes between the years. Mean yield lost to disease was 24.7% in 2012 and 19.3% in 2014. Despite the lack of relationship between seasons across all genotypes, analysis of the Lynx x Cadenza DH lines separately displayed a significant relationship in yield loss between years (p=0.005, r^2=0.598). The L14 x Rialto genotypes were more variable and did not show a significant relationship in yield loss between seasons (Fig. 4.13).

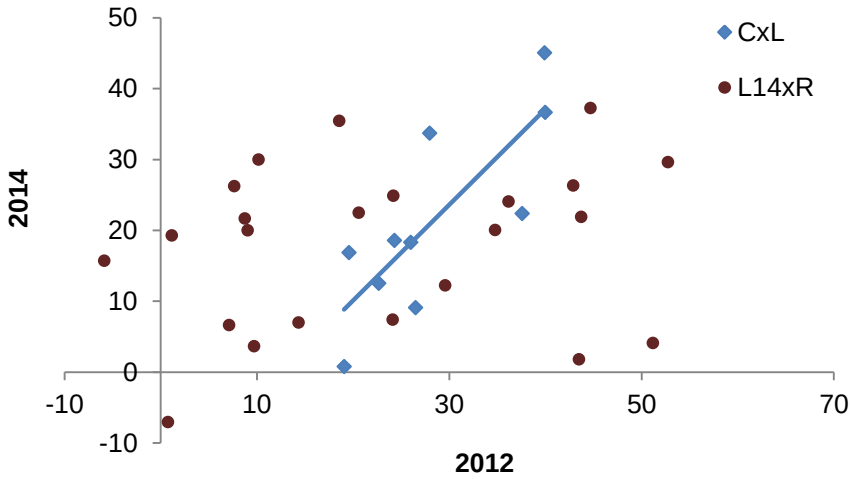


Fig 4.13. The relationship between grain yield loss (%) in 2012 and 2014. The two mapping populations, Cadenza x Lynx (r^2:0.598, P=0.005) and L14 x Rialto (p=NS), are shown separately.

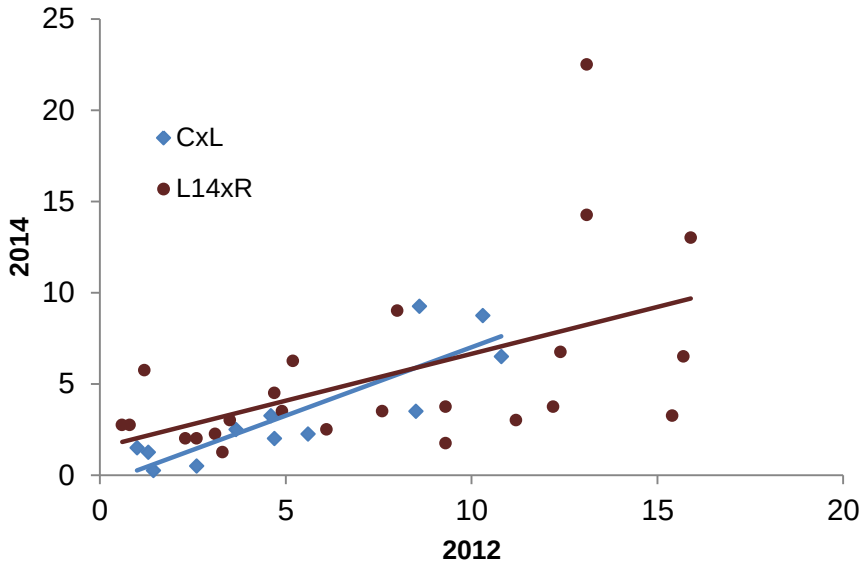
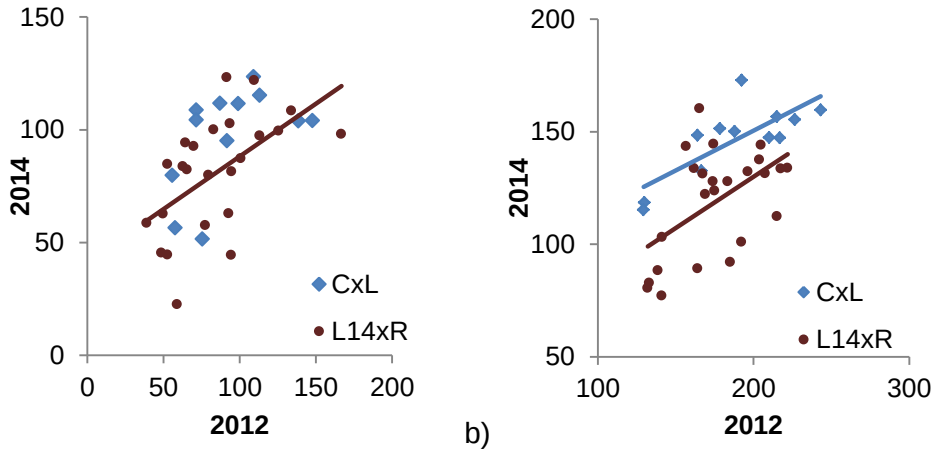


Fig 4.14. The relationship between the flag-leaf disease severity (%) at GS59 + 14 days in 2012 and 2014. The two mapping populations, Cadenza x Lynx (r^2:0.72) and L14 x Rialto (r^2:0.29), are shown separately.

There was a trend for disease severity to differ between years (p=0.062); the untreated flag-leaf disease at GS59 +14 days was 6.8% in 2012 and 4.8% in 2014 for the 12 common genotypes (Table 4.14). There was a relationship in disease severity between the two years (p<0.001, r^2=0.345) for all genotypes (Fig 4.14). In addition, there were relationships between the years for the separate mapping populations; but again the Cadenza x Lynx lines (p<0.001, r^2=0.717) displayed higher consistency across years than the L14 x Rialto lines (p=0.008, r^2=0.244).

There was no significant difference in untreated healthy area duration between the years; in 2012 the untreated HAD was 87.1 and 86.3 in 2014 (Table 4.13). However, the 2012 season had a considerably higher protected HAD than in 2014, 180.0 compared to 128.1 (p=0.007; Table 4.14). There were significant year x genotype interactions for both untreated and protected HAD (Table 4.14). There were relationships between HAD in the two years in both the untreated (p<0.001, r^2=0.294) and protected treatment (p<0.001, r^2=0.323, Fig 4.15). In the protected treatment, there were linear relationships between the two years for the separate groups (Fig. 4.15b), but there was only

a significant relationship between untreated HAD in the two years in the L14 x Rialto DH lines (p=0.005, r^2=0.279; Fig 4.15a).



a) b)

Fig 4.15. The linear relationship between untreated healthy area duration (HAD) amongst 12 genotypes between the field seasons 2012 and 2014. A) Untreated HAD (p<0.001, r^2=0.294) with Cadenza x Lynx (nonsignificant; r^2: 0.21) and L14 x Rialto DH lines (p=0.005; r^2:0.28) shown separately. B) Protected HAD (p<0.001, r^2=0.323) with Cadenza x Lynx (p=0.003; r^2:0.56) and L14 x Rialto crosses (p=0.008; r^2: 0.25) shown separately.

The largest difference between seasons was found in disease tolerance. There were shallower disease tolerance slopes (i.e. greater tolerance) in 2012 than in 2014 (p<0.001). Most lines followed this trend, except for L14xR133 which had increased tolerance in 2014 compared to 2012 (Table 4.14). Despite the large differences, there was a linear relationship between the two years (p<0.001, r^2=0.489). Analysis of the L14 x Rialto population showed a relationship between the seasons (p<0.001, r^2=0.412), but there was a much higher correlation in the Cadenza x Lynx population (p<0.001, r^2=0.983, Fig 4.16).

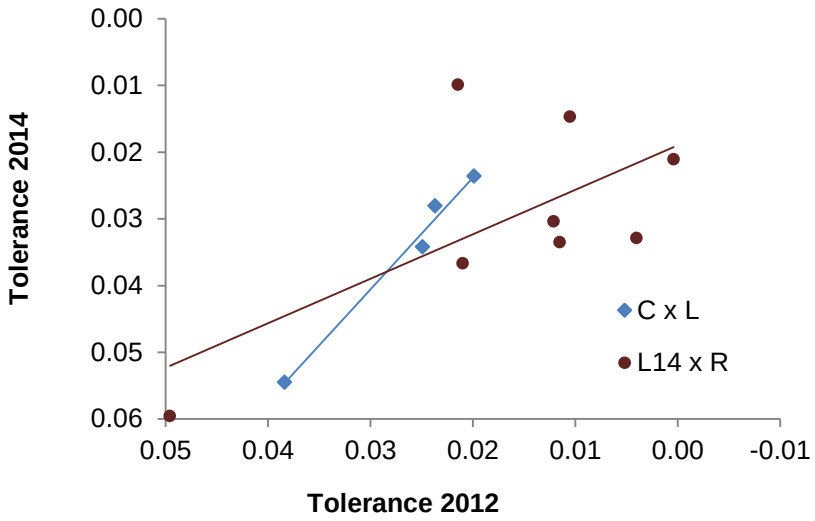


Fig 4.16. The linear regression amongst genotypes between 2012 and 2014 tolerance (t ha^{-1} HAD^{-1})values ($p<0.001$, $r^2=0.489$). The two DH populations, Cadenza x Lynx (r^2:0.98, $P<0.001$) and L14 x Rialto (r^2:0.41; $p<0.001$), are shown separately.

Table 4.14. The tolerance slopes, untreated and protected HAD, untreated and protected grain yield in the common genotypes in 2012 and 2014. The results of ANOVA are displayed below, reporting p-values below 0.1.

	Tolerance t ha^{-1} HAD^{-1}		**Untreated HAD**		**Protected HAD**		**Untreated yield t ha^{-1}**		**Protected yield t ha^{-1}**	
Genotype	**2012**	**2014**	**2012**	**2014**	**2012**	**2014**	**2012**	**2014**	**2012**	**2014**
CxL 14B	0.020	0.024	123.3	114.4	197.1	147.7	7.4	8.1	9.5	9.0
CxL 43A	0.025	0.034	85.8	110.7	199.1	159.9	6.6	5.8	9.6	7.5
CxL 5H	0.024	0.028	100.5	101.2	215.3	155.5	7.3	7.9	9.0	9.4
CxL 7A	0.038	0.054	62.9	62.7	141.9	122.2	3.9	5.0	6.5	8.5
L14xR 114	0.010	0.015	116.9	110.5	166.0	134.4	6.8	6.8	7.6	7.6
L14xR 127	0.012	0.034	90.2	69.4	153.0	84.1	7.8	6.0	7.8	7.5
L14xR 133	0.021	0.010	73.9	86.6	195.7	130.7	6.4	7.2	9.1	7.7
L14xR 16	0.021	0.037	65.7	82.4	199.1	133.9	5.0	6.8	8.1	8.8
L14xR 19	0.004	0.033	67.6	43.4	182.8	105.6	5.9	5.1	6.7	7.2
L14xR 20	0.000	0.021	88.6	96.8	181.1	147.6	5.3	6.6	5.5	7.7
L14xR 22	0.050	0.060	46.6	49.6	144.9	86.1	5.1	5.4	9.8	7.6
L14xR 92	0.012	0.030	123.2	107.7	184.2	130.0	7.1	7.3	8.5	7.9
Grand Total	**0.020**	**0.028**	**87.1**	**86.3**	**180.0**	**128.1**	**6.2**	**6.5**	**8.2**	**8.0**
P year	<0.001		*		*		*		*	
sed; df	0.0023; 142									
P genotype	*		<0.001		<0.001		<0.001		<0.001	
sed; df			6.73; 44		8.05; 44		0.24; 44		0.59; 43	
P year x genotype	*		0.015		0.022		<0.001		0.006	
sed; df			9.52; 44		11.38; 44		0.34; 44		0.84; 43	

4.4 Discussion

The main aim of this chapter was to quantify genetic variation in disease tolerance over two field seasons. Septoria leaf blotch disease had a significant impact on yield in both 2012 and 2014. This was expected and Septoria disease-yield relationships have been extensively reported, often as linear relationships, which was also the case in these experiments (Ziv and Eyal, 1978, Thomas et al., 1989). There were significant correlations between disease and grain yield at GS59 in both 2012 and 2014, but the relationship was strongest for flag-leaf disease at GS59+14 days, which was chosen as a representative assessment for disease resistance and susceptibility for the lines in regression analyses with yield components and disease tolerance scores.

There have been repeated reports of *Z. tritici* causing thousand grain weight reduction (Simón et al., 2002, Robert et al., 2004) and this was also the case in this study, although the relationships between STB and reduced TGW were quite weak. The effect of genetic variation of STB on yield was mainly conferred by decreased grains per m^2 for both these years. As there were no significant differences between fungicide treatments for ears per m^2, it is likely that this was conferred through a reduction in grains per ear. This is somewhat unexpected as wheat yields in climates such as UK and Ireland are generally more affected by post-anthesis restriction of green area (causing grain weight reduction) compared to pre-anthesis effects (Beed et al., 2007). Grains per m^2, on the other hand, is determined pre-anthesis until mid-flowering– normally before STB reaches the top two leaves. Despite the expectation that the major part of yield loss would be conferred through TGW, previous studies have shown STB to affect grains ear^{-1}. For instance, Simón et al. (2002) found a reduction of 6-22% in grains ear^{-1} due to STB in Argentinian field experiments. In France, Robert et al. (2004) found grains per ear reduced by 11-24 % in winter wheat cultivars Soissons and Recital, but did not separate effects of leaf rust and STB.

That the major part of yield loss is conferred through a reduction in grains per ear is a plausible explanation for the 2012 field experiment in which there was a 19% reduction in grains ear^{-1} when unprotected against STB. It is more difficult to conclude about the 2014 experiment as there were no

fungicide effects on ears per m^2 or on the number of grains per ear. It should, however, be noted that the analysis of the grain components was carried out on combine harvested samples. There is a possibility that the smallest grains were lost during harvest, which would increase the apparent TGW and decrease grains m^{-2} (Sylvester-Bradley et al., 1999). This would in turn disguise the effects of TGW on yield.

Necrotic leaf pathogens, such as *Z. tritici,* cause yield loss though loss of green area and consequently by decreased absorption of photosynthetically active radiation, PAR (Paveley, 1999). In agreement with this, the post-anthesis HAD values were strongly correlated with yield amongst genotypes in these field experiments. This was expected as HAD is reduced by green area loss caused by disease (Bryson et al., 1997, Parker et al., 2004). Furthermore, the relationship between HAD and TGW was stronger than the STB (at a single assessment) - TGW relationship. This was also expected as HAD is an integration of green area lost during the entire grain-filling period, and would therefore have a direct influence on TGW. However, the relationship between post-anthesis HAD and grains m^{-2} was stronger than the HAD - TGW relationship. As mentioned previously, grains m^{-2} is determined before the start of the grain-filling period, so a direct relationship between post-anthesis HAD and grains m^{-2} is unlikely. A plausible explanation is that there is a relationship between a large pre-anthesis green area, possibly through increased shoot numbers and hence ears m^{-2}, and a high post-anthesis HAD. There was little evidence to suggest that there was a higher ear number in plots with higher GLAI. However, there were strong relationships between HAD and GLAI at ear emergence.

There was a large range in disease tolerance in 2012 and 2014; at 4.4 and 3.8 standard deviations they were larger than previously reported genetic ranges in disease tolerance. For instance, Parker et al. (2004) studied 25 UK varieties over several sites and seasons and found a tolerance range of 3.2 standard deviations. In 2012, the most tolerant genotype had a negative slope of -0.001 t ha^{-1} HAD, i.e. a slightly higher untreated than fungicide protected yield. The most intolerant genotype had a slope of 0.050 t ha^{-1} HAD and had lost nearly half its yield (9.8 to 5.1 t ha^{-1}) to disease, and had approximately 50 times more yield loss per unit green area loss than the most tolerant genotype. The difference between the most tolerant and the most intolerant genotype was even larger in 2014, the most tolerant genotype had a slope of

0.00 and the most intolerant a slope of 0.06 t ha^{-1} HAD^{-1}. In comparison, the slopes found by Parker et al. (2004) had a range of 0.0084 t ha^{-1} HAD^{-1}; but this may have been due to a lack of genotypes with extreme high/low disease tolerance.

The extended disease tolerance ranges in these experiments are primarily due to an increase in disease tolerance, conferred by the CIMMYT-derived doubled-haploid lines. All lines displaying significant tolerance in 2012 belonged to the L14 x Rialto population and the same was true for 2014, with the addition of one L8 x Rialto line. In fact, in 2012 more than 50% of the L14 x Rialto population displayed disease tolerance while over 70% of genotypes with UK background were significantly intolerant. This trend was less extreme in the 2014 experiment due to a smaller number of genotypes and consequently less significant high/low tolerance. However, two out of the three intolerant genotypes had a UK background. This supports the argument that in order to find novel tolerance traits, there might be a need to introduce a wider genetic diversity into existing UK germplasm. As previously mentioned, the reference lines used in these experiments displayed tolerance/intolerance rankings in the expected order. However, the reference lines that had previously displayed tolerance were not significantly tolerant compared to the rest of the genotypes. In fact, some of these were classified as significantly intolerant in 2012 (Table 4.11). Disease tolerance, however, is a comparative scale, which means that genotypes that are not significantly tolerant in one experiment might be tolerant in comparison to other genotypes.

Many studies have shown a relationship between high disease tolerance and low yield potential (Bingham et al., 2009) and one of the aims of this study was to determine if disease tolerance and yields are necessarily linked. Even though there was a relationship between high disease tolerance and low yield in 2012, it was quite weak, and many of the lines did not conform to this relationship. When selecting genotypes for the in-depth studies (see Chapter 5), particular care was taken to include genotypes displaying disease tolerance and relatively high yield potential as highlighted in Fig 4.17. Furthermore, there was no relationship between disease tolerance and protected yield in 2014. This indicates that genetic variation in disease tolerance can be identified independent of yield potential.

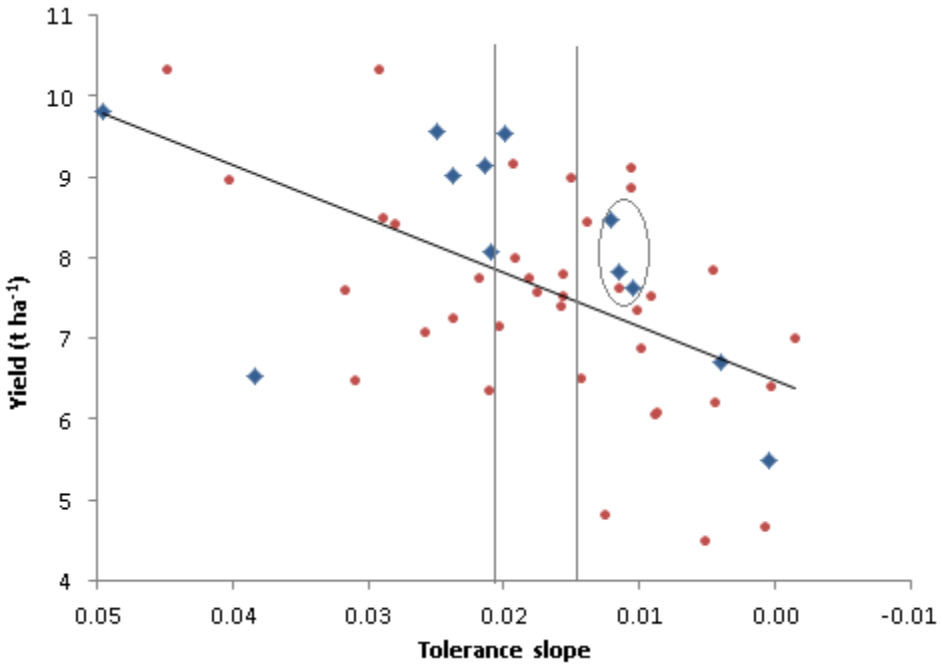


Fig 4.17. The relationship between disease tolerance (t ha^{-1} HAD^{-1}) and protected yield in 2012. The 95% confidence intervals for disease tolerance are shown as grey vertical lines. The twelve genotypes common in 2012 and 2014 are shown as blue squares, and three of these (L14 x R114, -127, and -92) displaying high tolerance and high yield potential are circled.

One of the major challenges of identifying disease tolerance traits is to distinguish disease tolerance from disease resistance and escape (Bingham et al., 2009, Ney et al., 2013). Present results showed a very weak, but significant, relationship between crop height and flag-leaf disease severity in 2012. Crop height only accounted for 5% of the genetic variation of flag-leaf disease severity, but results still indicate that disease escape might influence the disease severity. In 2014, there was a stronger crop height influence that accounted for nearly 20% of the variation in flag-leaf disease severity. However, as no correlation was found between crop height and disease tolerance the disease tolerance calculation is not presently adjusted for crop height.

There were clearly large differences in disease resistance in the 2012 field experiment. However, disease resistance did not seem to affect the tolerance scores as there were no significant differences in disease severities between the tolerant and intolerant genotypes. In contrast, there were indications that disease resistance could be influential in the 2014 experiment

as the intolerant genotypes displayed significantly higher flag-leaf disease severity than the tolerant and “neutral” genotypes. However, due to the small numbers of lines in 2014 it is difficult to determine with certainty if there are relationships between disease tolerance, escape and resistance. The difficulty of separating disease tolerance and resistance is not unusual, for instance Politowski and Browning (1978) discussed the differences between “true tolerance” and what they termed “dilatory resistance” (the reduction of pathogen development, currently known as partial resistance). They highlighted the importance of several specific disease assessments throughout the season, in order to determine true tolerance.

In order for disease tolerance to be incorporated into breeding programmes, any genotype needs to display disease tolerance over different seasons. In general, the four Cadenza x Lynx genotypes displayed high stability between years; especially in disease tolerance. This was expected as these genotypes have been bred for yield stability in the UK, and have been used as reference lines in this and previous tolerance experiments (Julie Smith, pers. comm.). The L14 x Rialto genotypes were generally less stable over site-seasons as a population, but nevertheless showed stability in disease tolerance levels. In particular, L14 x R114 and L14 x R20 displayed significant disease tolerance in both 2012 and 2014. Similarly, L14 x R22 displayed the highest intolerance in both years. On the other hand, L14 x R133 was significantly intolerant in 2012 but displayed high tolerance in 2014, which introduces some uncertainty into the results. The differences between site-seasons can to some extent be explained by site variation. It is generally accepted that tolerance of genotypes will interact with environment. For instance, Israeli spring wheat displaying disease tolerance in Israel (Zuckerman et al., 1997) did not display tolerance in the UK (Julie Smith, pers. comm.). Differences in wheat fitness traits between environments has been extensively reported, primarily in grain yield (Baker, 1969, Crossa et al., 1991, Trethowan and Crossa, 2007).

4.5 Conclusions

The results from this study indicate that it is possible to increase the range of disease tolerance by introgressing exotic germplasm into UK adapted material. This is mainly shown by CIMMYT spring wheat x UK winter wheat Rialto crosses displaying a wider range of disease tolerance than UK

genotypes. Disease tolerance, and the traits tolerance is calculated from, also seems to be moderately heritable. However, mapping populations might need several years of screening in order to select stable genotypes for in-depth studies on STB tolerance mechanisms and for pre-breeding for STB tolerance. Encouragingly, the results from this study indicate that the association between high disease tolerance and low yield potential can be broken. Mechanisms associated with STB disease tolerance will be further investigated in Chapter 5, in order to identify targets for selection criteria in wheat breeding.

5. Physiological traits conferring disease tolerance in field experiments

5.1 Introduction

Disease tolerance, the ability to maintain grain yield in the presence of disease, has been observed for cereal crops repeatedly over the last century with observations as early as 1894 (Schafer, 1971, Parker et al., 2004). Although disease tolerance has been observed for some time, it remains the least well known plant defence mechanism (particularly in comparison with disease resistance). A number of traits have been suggested as potentially conferring disease tolerance, but few of these have been supported by experimental data in several independent studies.

The majority of putative tolerance traits are related to resource use, resource capture and high source to sink balance (Paveley et al., 2008, Bingham et al., 2009). For instance, the ability to maintain (or increase) radiation-use efficiency in the presence of disease has been suggested as a mechanism to buffer loss of green area (Bingham et al., 2009). A large green area index (and flag leaf size) could maintain a high fractional interception (i.e. radiation capture). Similarly, a high light extinction coefficient increases fractional radiation interception per unit green area and radiation capture in the upper canopy, which has prolonged green area duration (Parker et al., 2004, Paveley et al., 2008, Bingham et al., 2009). A higher source to sink balance in the absence of disease could buffer the loss of source capacity caused by disease and reduce the yield loss per unit green area to disease (Paveley et al., 2008). Additional hypotheses regarding resource use suggest that tolerant varieties have larger stem water soluble carbohydrate (WSC) storage capacity that subsequently buffers the loss of green area caused by disease (Parker et al., 2004, Foulkes et al., 2006).

In order for disease tolerance to be incorporated into modern wheat breeding it is important to identify heritable traits conferring disease tolerance that a) can easily be used for screening in breeding programs and b) are not coupled with low yield potential. In order to test these putative tolerance traits,

in-depth phenotyping was performed on 16 genotypes selected according to previous contrasting displays of high/low disease tolerance.

The specific hypotheses tested with regard to genetic variation in this chapter are:

1. An increase of green canopy area at ear emergence will be beneficial for disease tolerance as for a given level of disease severity there will be a larger amount of photosynthetic area remaining post-infection to maintain radiation interception.
2. A higher light extinction coefficient will be beneficial to disease tolerance, as it would increase light interception in the top two leaves relative to lower leaves, due to the typically greater disease severity in the lower canopy having a lesser opportunity to impact on radiation interception and yield.
3. A high source to sink balance as indicated by post-anthesis healthy area duration (HAD) per grain in uninfected crops is beneficial for disease tolerance.
4. A high capacity for water soluble carbohydrate (WSC) stem storage is beneficial for disease tolerance as a buffer for loss of photosynthetic capacity due to disease-induced green area loss during grain filling.

5.2 Materials and methods

A field experiment was conducted in 2013/2014 in Hereford, UK, in which 16 lines selected from three doubled-haploid populations were phenotyped. The majority of the phenotyping results presented are from this experiment, but data from the 2011/2012 field experiment in Carlow, Ireland (49 genotypes) has also been included for traits that were commonly examined in both years.

The field experiments were conducted as split plot designs in three replicate blocks, with fungicide treatments (untreated and protected against *Zymoseptoria tritici*) as main plots and genotypes as sub-plots. The key traits measured were green area, light interception, above-ground biomass (pre-anthesis, at anthesis and at physiological maturity), radiation-use efficiency and WSC stem storage. Within each site-season, each physiological trait was analysed by split-plot ANOVA on the full range of genotypes (unless stated otherwise). For those traits which were studied in both 2012 and 2014, a cross-year analysis was performed for the 12 common genotypes by linear mixed model regression analysis (REML). The genotypes were grouped into "CIMMYT lines" (L14/L8 x Rialto DH lines) and "UK lines" (Rialto, Lynx x Cadenza and Avalon x Cadenza DH lines) in order to compare differences in traits between exotic germplasm and UK-derived germplasm. The differences between these two groups were determined by one-way ANOVAs.

Full details of the sites, experimental treatments and disease and crop measurements are reported in chapter 3, but to summarise;

- HAD (Healthy area duration) was integrated over the top 4 leaves from leaf area measurements and green leaf area percentage assessments carried out at growth stages 59, 59 + 14d and 59 + 28d and the final senescence score.
- Green lamina area index (GLAI) and leaf area index (LAI) were determined at GS59 over the top 4 leaves.
- Disease tolerance was calculated as the slope between HAD and grain yield from both fungicide treatments.

5.3 Results

5.3.1 Flag leaf traits and canopy size

Flag-leaf area (FLA) was measured at ear emergence in 2012 and 2014. There was no significant difference in FLA between years when the 12 genotypes present in 2012 and 2014 field experiments were analysed over both seasons. Total genotype (G) range for FLA in 2012 was from 26.2 to 47.3 cm^2 ($p<0.001$; Table 5.1) whilst the range for the same genotypes grown during 2014 was 32.5 to 47.1 cm^2, compared to 28.3 to 48.5 cm^2 for all genotypes in 2014 ($p<0.001$). There was no fungicide treatment (FT) effect or FT x G interaction in either year. There was a strong year (Y) x G interaction ($p<0.001$) but no Y x FT x G interaction (Table 5.1). Given the strong Y x G interaction there was only a weak relationship between flag-leaf area in 2012 and 2014 ($p=0.011$, $r^2=0.077$) (Fig 5.1a). There was no relationship between FLA and disease tolerance, or FLA and protected yield in either year (Table 5.1).

The flag-leaf length at ear emergence in 2012 varied from 16.4 to 24.3 cm between genotypes (L14xR43 – CxL14B; $p<0.001$). There was no FT effect or FT x G interaction. In 2014, there were again large variations in flag-leaf length between genotypes, between 20.8 and 27.5 cm (L8xR86 - AxC21; $p<0.001$). There was a trend for a difference between FT ($p=0.068$), but no FT x G interaction during 2014 (Table 5.2). Flag-leaves were longer in 2014 than in 2012, both for the total populations (24.2 vs. 20.7 cm) and for the 12 common genotypes (24.2 vs. 21.5 cm). Fungicide protected flag leaves tended to be shorter than untreated flag leaves in 2014 but there was no FT effect in 2012. There was also a significant Y x G interaction ($p=0.001$) resulting in only a weak but significant relationship between the flag-leaf lengths in 2012 and 2014 for the 12 genotypes ($p=0.002$, $r^2=0.121$) (Fig 5.1b). There were no FT x G or Y x FT x G interactions (Table 5.2). There was no relationship between disease tolerance and flag-leaf length in 2012 or 2014 and there was no relationship between protected grain yield and flag leaf length in either treatment.

Table 5.1. Flag leaf area (cm^2) in the field experiments 2012 and 2014. Reported are significant ($p<0.05$) and near-significant ($p<0.1$) values.

Genotype	2012 untr.	2012 prot.	2014 untr.	2014 prot.
AxC 117	-	-	38.9	39.2
AxC 21	-	-	46.3	45.5
CxL 14B	41.2	39.9	37.2	39.0
CxL 3D	39.6	39.7	-	-
CxL 41F	38.3	37.4	-	-
CxL 42C	38.4	37.6	-	-
CxL 43A	34.5	30.5	41.8	41.4
CxL 48D	38.3	32.9	-	-
CxL 5H	38.5	43.1	34.3	34.3
CxL 6E	34.7	34.7	-	-
CxL 7a	40.4	40.3	36.9	39.7
CxL 8I	38.0	40.1	-	-
L14 x R 10	40.8	42.1	-	-
L14 x R 104	35.9	36.0	-	-
L14 x R 107	35.8	37.4	-	-
L14 x R 112	40.5	46.1	-	-
L14 x R 114	39.2	39.8	45.8	46.3
L14 x R 119	37.9	40.8	-	-
L14 x R 121	40.2	43.7	-	-
L14 x R 123	37.5	39.0	-	-
L14 x R 127	33.7	34.8	34.6	31.4
L14 x R 129	33.0	28.5	-	-
L14 x R 131	37.1	34.2	-	-
L14 x R 133	41.6	47.5	47.3	40.9
L14 x R 136	40.1	37.8	-	-
L14 x R 138	47.1	47.6	-	-
L14 x R 15	40.2	40.1	-	-
L14 x R 16	45.9	48.2	45.2	51.9
L14 x R 19	39.4	36.9	36.3	39.1
L14 x R 20	40.9	46.2	43.5	47.1
L14 x R 22	38.1	39.9	39.7	38.7
L14 x R 23	41.0	43.5	-	-
L14 x R 35	39.1	40.9	-	-
L14 x R 43	24.6	27.7	-	-
L14 x R 5	42.4	42.1	-	-
L14 x R 50	34.4	33.0	-	-
L14 x R 55	43.1	45.3	-	-
L14 x R 59	41.4	39.6	-	-
L14 x R 62	36.3	37.7	-	-
L14 x R 66	38.5	37.9	-	-
L14 x R 68	46.7	43.5	-	-
L14 x R 7	41.5	42.7	-	-
L14 x R 70	40.8	37.2	-	-
L14 x R 74	38.0	37.9	-	-
L14 x R 80	33.6	33.4	-	-
L14 x R 82	39.6	36.0	-	-
L14 x R 85	38.4	39.5	-	-
L14 x R 89	37.4	37.9	-	-
L14 x R 91	38.0	37.3	-	-
L14 x R 92	41.3	41.1	44.4	38.3
L8xR 10	-	-	37.3	34.2
L8xR 86	-	-	28.8	27.7
Rialto	34.9	37.5	-	-
Averages	**39.1**	**38.9**	**39.7**	**39.8**
12 common genotypes	39.6	40.7	40.6	40.7
CIMMYT	39.0	39.5	40.3	39.6
UK	37.9	37.6	33.2	39.9
P Fungicide (SED; df)	NS		NS	
P Genotype (SED; df)	<0.001	(2.401; 192)	<0.001	(2.300; 60)
P FT x G (SED; df)	NS		NS	
P FT (SED; df)	0.067	(2.97; 4)		
P G (SED; df)	<0.001	(3.08;88)		
P Y x FT (SED; df)	0.096	(4.20; 4)		
P Y x G (SED; df)	<0.001	(4.35; 88)		
P FT x G (SED; df)	NS			
P Y x FT x G (SED; df)	NS			
Regression				
P tolerance r^2, direction	NS	NS	NS	NS
P protected yield r^2, direction	-	NS	-	NS

Table 5.2. Flag leaf length (cm) in in the field experiments 2012 and 2014. Reported are significant (p<0.05) and near-significant (p<0.1) values.

Genotype	2012 untr.	2012 prot.	2014 untr.	2014 prot.
AxC 117	-	-	25.2	24.4
AxC 21	-	-	27.9	27.0
CxL 14B	24.5	24.2	25.3	26.4
CxL 3D	22.3	22.9	-	-
CxL 41F	20.6	20.6	-	-
CxL 42C	22.0	20.7	-	-
CxL 43A	20.8	17.9	26.1	24.7
CxL 48D	22.1	20.3	-	-
CxL 5H	21.7	23.1	22.3	22.3
CxL 6E	17.8	17.9	-	-
CxL 7a	22.2	21.6	23.9	23.5
CxL 8I	21.2	21.6	-	-
L14 x R 10	21.2	20.4	-	-
L14 x R 104	17.3	17.1	-	-
L14 x R 107	19.4	19.7	-	-
L14 x R 112	22.7	25.4	-	-
L14 x R 114	21.1	21.5	27.4	25.5
L14 x R 119	20.2	20.0	-	-
L14 x R 121	20.2	21.3	-	-
L14 x R 123	20.2	20.9	-	-
L14 x R 127	18.8	17.2	21.9	20.4
L14 x R 129	19.1	17.5	-	-
L14 x R 131	20.1	19.5	-	-
L14 x R 133	21.8	23.2	26.2	23.7
L14 x R 136	21.8	20.4	-	-
L14 x R 138	20.4	21.3	-	-
L14 x R 15	21.8	21.8	-	-
L14 x R 16	23.5	23.5	26.5	26.4
L14 x R 19	19.5	17.8	21.0	20.5
L14 x R 20	21.4	21.9	24.6	23.8
L14 x R 22	21.8	22.0	24.9	24.0
L14 x R 23	21.0	21.3	-	-
L14 x R 35	20.6	21.4	-	-
L14 x R 43	15.8	17.0	-	-
L14 x R 5	22.1	22.0	-	-
L14 x R 50	18.9	19.2	-	-
L14 x R 55	22.1	22.5	-	-
L14 x R 59	22.3	21.6	-	-
L14 x R 62	19.5	19.8	-	-
L14 x R 66	20.0	18.9	-	-
L14 x R 68	21.9	20.4	-	-
L14 x R 7	24.1	24.3	-	-
L14 x R 70	18.7	18.1	-	-
L14 x R 74	21.4	20.9	-	-
L14 x R 80	19.5	19.3	-	-
L14 x R 82	19.5	18.7	-	-
L14 x R 85	21.0	20.1	-	-
L14 x R 89	22.6	22.1	-	-
L14 x R 91	18.9	19.4	-	-
L14 x R 92	22.5	22.4	26.5	23.1
L8xR 10	-	-	23.6	21.9
L8xR 86	-	-	21.3	20.3
Rialto	18.1	19.0	-	-
Average	**20.8**	**20.6**	**24.7**	**23.6**
12 common genotypes	21.6	21.4	24.7	23.7
CIMMYT	20.8	20.6	24.4	25.1
UK	21.2	20.9	23.0	24.7
P Fungicide (SED; df)	NS		0.068	(0.284; 2)
P genotype (SED; df)	<0.001	(0.916; 192)	<0.001	(1.002; 60)
P FT x G (SED; df)	NS		NS	
P FT (SED; df)	0.003	(1.22; 4)		
P G (SED; df)	<0.001	(1.27; 88)		
P Y x FT (SED; df)	0.017	(1.72; 4)		
P Y x G (SED; df)	0.001	(1.79; 88)		
P FT x G (SED; df)	NS			
P Y x FT x G (SED; df)	NS			
Linear regression				
P tolerance r^2, direction	NS	NS	NS	NS
P protected yield r^2, direction	-	NS	-	NS

Flag-leaf width in 2012 of all evaluated genotypes ranged from 1.92 to 2.73 cm (L14xR43 - L14x138; $p<0.001$). There was no difference between FT ($p=0.16$) and no FT x G interaction (Table 5.3). In 2014, there was a trend for FT to increase leaf width ($p=0.059$) and a FT x G interaction ($p=0.056$). The average leaf width increased from 1.94 cm untreated to 2.02 cm in the fungicide protected treatment, while the genotypes ranged between 1.63 and 2.17 cm when untreated and 1.65 and 2.38 cm under fungicide protection. There was no significant relationship between flag-leaf width and disease tolerance, or between flag-leaf width and untreated or protected grain yield. A cross-year analysis on the 12 common genotypes displayed a significant year ($p=0.007$) and fungicide effect ($p=0.002$), but no Y x FT interaction. The flag leaves were generally wider in 2012 than in 2014 (2.25 vs. 2.02 cm, respectively) and the leaves were wider when fungicide protected compared to untreated both years (Table 5.3). The cross-year analysis indicated a Y x G interaction ($p=0.009$) and trends for FT x G and Y x FT x G interactions ($p=0.096$ and 0.077, respectively; Table 5.3). There was a stronger relationship between 2012 and 2014 leaf widths than leaf length or area ($p<0.001$, $r^2=0.269$) (Fig. 5.1c). With the exception of a few genotypes the ranking for leaf widths followed a similar pattern in both years.

Flag-leaf specific dry weight (SDW; g m^{-2}) was measured at GS59 in fungicide-protected plots only (Table 5.4). In 2012 flag-leaf SDW varied between genotypes from 50.9 to 74.6 g m^{-2} (L14xR114 - L14xR80; $p<0.001$; Table 5.4). There was no relationship between genetic variation in flag-leaf SDW and disease tolerance in 2012. In 2014, there were no differences between genotypes and no relationship between flag-leaf SDW and disease tolerance. The cross-year analysis of the common genotypes showed a genotype effect ($p=0.023$) but no year effect and no Y x G interaction.

Table 5.3. Flag leaf width (cm) in the field experiments 2012 and 2014. Reported are significant (p<0.05) and near-significant (p<0.1) values.

Genotype	**2012 untr**	**2012 prot**	**2014 untr**	**2014 prot**
AxC 117	-	-	1.86	1.93
AxC 21	-	-	1.99	2.03
CxL 14B	2.03	2.0	1.77	1.77
CxL 3D	2.14	2.1	-	-
CxL 41F	2.23	2.2	-	-
CxL 42C	2.10	2.2	-	-
CxL 43A	2.00	2.1	1.93	2.01
CxL 48D	2.09	2.0	-	-
CxL 5H	2.14	2.3	1.85	1.85
CxL 6E	2.34	2.3	-	-
CxL 7a	2.20	2.3	1.87	2.03
CxL 8I	2.16	2.2	-	-
L14 x R 10	2.31	2.5	-	-
L14 x R 104	2.50	2.5	-	-
L14 x R 107	2.22	2.3	-	-
L14 x R 112	2.15	2.2	-	-
L14 x R 114	2.23	2.2	2.01	2.19
L14 x R 119	2.26	2.5	-	-
L14 x R 121	2.40	2.5	-	-
L14 x R 123	2.23	2.3	-	-
L14 x R 127	2.17	2.4	1.89	1.85
L14 x R 129	2.08	2.0	-	-
L14 x R 131	2.23	2.1	-	-
L14 x R 133	2.30	2.5	2.17	2.07
L14 x R 136	2.21	2.2	-	-
L14 x R 138	2.78	2.7	-	-
L14 x R 15	2.23	2.2	-	-
L14 x R 16	2.36	2.5	2.04	2.37
L14 x R 19	2.44	2.5	2.09	2.29
L14 x R 20	2.30	2.5	2.13	2.38
L14 x R 22	2.11	2.2	1.92	1.94
L14 x R 23	2.36	2.5	-	-
L14 x R 35	2.28	2.3	-	-
L14 x R 43	1.88	2.0	-	-
L14 x R 5	2.29	2.3	-	-
L14 x R 50	2.19	2.1	-	-
L14 x R 55	2.35	2.4	-	-
L14 x R 59	2.23	2.2	-	-
L14 x R 62	2.23	2.3	-	-
L14 x R 66	2.32	2.4	-	-
L14 x R 68	2.56	2.6	-	-
L14 x R 7	2.08	2.1	-	-
L14 x R 70	2.62	2.5	-	-
L14 x R 74	2.13	2.2	-	-
L14 x R 80	2.07	2.1	-	-
L14 x R 82	2.45	2.3	-	-
L14 x R 85	2.20	2.4	-	-
L14 x R 89	1.99	2.1	-	-
L14 x R 91	2.42	2.3	-	-
L14 x R 92	2.21	2.2	2.02	2.00
L8xR 10	-	-	1.91	1.88
L8xR 86	-	-	1.63	1.65
Rialto	2.32	2.4	-	-
Average	**2.25**	**2.28**	**1.94**	**2.02**
12 common genotypes	2.21	2.30	1.98	2.06
CIMMYT	2.27	2.31	1.98	2.06
UK	2.16	2.17	1.88	1.94
P Fungicide (SED; df)	NS	0.0157 (2)	0.059	(0.018; 2)
P genotype (SED; df)	<0.001	0.066 (192)	<0.001	(0.063; 60)
P FT x G (SED; df)	NS	0.0934 (192)	0.056	(0.089; 60)
P FT (SED; df)	0.002	(0.086; 4)		
P G (SED; df)	<0.001	(0.088; 88)		
P Y x FT (SED; df)	NS			
P Y x G (SED; df)	0.009	(0.125; 88)		
P FT x G (SED; df)	0.096	(0.125; 88)		
P Y x FT x G (SED; df)	0.007	(0.176; 88)		
Linear regression				
P tolerance r^2, direction	NS	NS	NS	NS
P protected yield r^2, direction	-	NS	-	NS

Table 5.4. Specific flag leaf dry weight (g m^{-2}) in the field experiments 2012 and 2014. Reported are significant (p<0.05) and near-significant (p<0.1) values for one way ANOVAs within each season, and across years for the 12 common genotypes (split plot ANOVA).

Genotype	**2012 prot**	**2014 prot**
AxC 117	-	5.21
AxC 21	-	4.67
CxL 14B	5.79	4.42
CxL 3D	6.16	-
CxL 41F	5.78	-
CxL 42C	5.69	-
CxL 43A	5.94	5.78
CxL 48D	5.26	-
CxL 5H	5.55	5.61
CxL 6E	5.94	-
CxL 7a	5.43	4.97
CxL 8I	5.33	-
L14 x R 10	6.55	-
L14 x R 104	6.30	-
L14 x R 107	6.10	-
L14 x R 112	10.35	-
L14 x R 114	5.09	4.85
L14 x R 119	6.22	-
L14 x R 121	5.94	-
L14 x R 123	5.42	-
L14 x R 127	5.86	5.86
L14 x R 129	6.14	-
L14 x R 131	5.62	-
L14 x R 133	6.20	5.87
L14 x R 136	5.90	-
L14 x R 138	7.32	-
L14 x R 15	6.09	-
L14 x R 16	6.17	5.12
L14 x R 19	6.04	5.67
L14 x R 20	5.62	5.49
L14 x R 22	5.97	5.54
L14 x R 23	5.66	-
L14 x R 35	5.86	-
L14 x R 43	6.54	-
L14 x R 5	6.02	-
L14 x R 50	6.12	-
L14 x R 55	5.67	-
L14 x R 59	6.44	-
L14 x R 62	6.80	-
L14 x R 66	5.89	-
L14 x R 68	5.19	-
L14 x R 7	6.40	-
L14 x R 70	5.61	-
L14 x R 74	6.30	-
L14 x R 80	7.46	-
L14 x R 82	6.44	-
L14 x R 85	6.13	-
L14 x R 89	5.84	-
L14 x R 91	6.22	-
L14 x R 92	5.72	5.65
L8xR 10	-	4.74
L8xR 86	-	5.38
Rialto	5.30	-
Average	**6.07**	**5.30**
12 common genotypes	5.78	5.40
CIMMYT	6.18	5.42
UK	5.65	5.11
ANOVA (total)		
P genotype (SED; df)	<0.001 (0.469 ; 95)	NS
ANOVA (cross year)		
P G (SED; df)	0.023 (0.309; 44)	
P Y x G (SED; df)	NS	
Linear regression		
P tolerance	NS	NS
r^2, direction		
P protected yield	0.058	NS
r^2, direction	0.019; (-)	

The average total leaf lamina area index (LAI) at ear emergence was 6.62 in 2012 and ranged from 4.82 to 8.05 (L14xR112 – L14xR70; $p<0.001$; Table 5.5). The average LAI was smaller in 2014 at 5.18 with a range from 4.12 to 5.96 (L14xR127 - CxL43A; $p<0.001$) amongst genotypes. There was no relationship between disease tolerance and LAI in 2012, but there was a weak positive relationship between LAI and protected yield ($p=0.003$, $r^2=0.056$). On the other hand, a large untreated LAI was related to increased tolerance in 2014 ($p=0.019$; $r^2=0.095$) but there was no significant relationship between protected LAI and disease tolerance or protected LAI and protected yield. The cross-year analysis showed differences between years, with approximately 1.6 units more LAI in 2012 compared to 2014 ($p<0.001$). There was no Y x FT interaction or Y x FT x G interactions (Table 5.4). Despite a strong Y x G interaction ($p<0.001$), there was a relationship between the LAI of the genotypes in the two years ($p<0.001$, $r^2=0.349$; Fig 5.1d) and the genotype rankings remained similar between years.

In 2012, the average fungicide-treated green lamina area index (GLAI) value was 5.34 while the untreated crops had a lower ($p=0.010$) GLAI of 4.36, with a significant FT x G interaction ($p<0.001$; Table 5.6). The range of GLAI amongst protected genotypes was 3.63 to 8.88 (L14xR112 - L14xR123) and 2.85 – 6.41 (L14xR112 – L14xR123) for untreated GLAI ($p<0.001$). There was no relationship between disease tolerance and GLAI in either treatment, but there were relationships between protected grain yield and the GLAI of protected plants ($p<0.001$, $r^2=0.143$). Similarly, a significant relationship was observed between and grain yield and GLAI for untreated plants ($p<0.001$, $r^2=0.257$; Table 5.6). In 2014, the average fungicide-protected GLAI was 4.79 and significantly lower in the untreated plots at 4.26 ($p=0.006$), with a trend indicating a FT x G interaction ($p=0.075$; Table 5.6). Protected GLAI ranged between 3.81 and 5.47 (L14xR22 - CxL5H) and untreated GLAI ranged 2.56 – 5.32 (L14xR19 – CxL43A; $p<0.001$). There was a positive relationship between untreated GLAI and disease tolerance in 2014 ($p=0.003$; $r^2=0.163$) and also between protected GLAI and tolerance ($p=0.019$, $r^2=0.095$). There was no relationship between protected GLAI and protected yield potential, but a positive relationship between untreated GLAI and untreated yield ($p<0.001$, $r^2=0.276$; Table 5.6).

Table 5.5. Leaf area index (m^2 leaf lamina for the top 4 leaf layers per m^2) at ear emergence in the field experiments 2012 and 2014. P-values below 0.1 are reported.

Genotype	2012 untr	2012 prot	2014 untr	2014 prot
AxC 117	-	-	5.23	5.45
AxC 21	-	-	5.45	5.54
CxL 14B	7.65	8.15	5.46	5.66
CxL 3D	6.46	6.52	-	-
CxL 41F	5.79	6.69	-	-
CxL 42C	6.32	6.96	-	-
CxL 43A	6.55	6.78	6.08	5.85
CxL 48D	6.05	6.14	-	-
CxL 5H	6.61	7.10	5.65	5.67
CxL 6E	5.90	6.17	-	-
CxL 7a	5.95	6.42	4.74	5.22
CxL 8I	6.42	6.92	-	-
L14 x R 10	7.33	6.07	-	-
L14 x R 104	5.32	5.76	-	-
L14 x R 107	6.00	6.73	-	-
L14 x R 112	4.55	5.00	-	-
L14 x R 114	7.92	7.55	5.57	5.72
L14 x R 119	7.36	7.74	-	-
L14 x R 121	6.65	7.02	-	-
L14 x R 123	9.08	9.72	-	-
L14 x R 127	5.25	5.83	4.09	4.15
L14 x R 129	5.98	5.36	-	-
L14 x R 131	5.98	5.36	-	-
L14 x R 133	6.15	6.26	5.03	4.60
L14 x R 136	6.81	7.68	-	-
L14 x R 138	7.21	7.04	-	-
L14 x R 15	6.33	6.24	-	-
L14 x R 16	6.54	7.37	5.08	5.54
L14 x R 19	6.51	6.45	4.53	4.90
L14 x R 20	6.49	7.39	5.09	5.39
L14 x R 22	5.71	5.55	4.37	4.51
L14 x R 23	6.84	7.38	-	-
L14 x R 35	6.72	6.93	-	-
L14 x R 43	4.91	5.34	-	-
L14 x R 5	7.04	6.82	-	-
L14 x R 50	7.14	6.52	-	-
L14 x R 55	6.36	6.66	-	-
L14 x R 59	5.87	5.42	-	-
L14 x R 62	*	6.52	-	-
L14 x R 66	6.37	6.89	-	-
L14 x R 68	7.73	7.53	-	-
L14 x R 7	7.40	7.46	-	-
L14 x R 70	8.13	7.98	-	-
L14 x R 74	5.95	6.17	-	-
L14 x R 80	5.73	6.11	-	-
L14 x R 82	8.01	7.77	-	-
L14 x R 85	6.35	6.56	-	-
L14 x R 89	6.23	6.36	-	-
L14 x R 91	6.39	5.99	-	-
L14 x R 92	6.53	6.79	5.34	5.10
L8xR 10	-	-	5.44	5.61
L8xR 86	-	-	4.89	4.75
Rialto	6.52	7.08	-	-
Average	**6.53**	**6.70**	**5.13**	**5.24**
12 common genotypes	6.53	6.80	5.09	5.20
CIMMYT	6.58	6.67	4.94	5.03
UK	6.40	6.81	5.43	5.59
P Fungicide (SED; df)	NS		NS	
P genotype (SED; df)	<0.001	(0.275; 168)	<0.001	(0.197; 58)
P FT x G (SED; df)	0.077	(0.388; 168)	NS	
P FT (SED; df)	0.014	(0.346; 4)		
P G (SED; df)	<0.001	(0.380; 81.5)		
P Y x FT (SED; df)	NS			
P Y x G (SED; df)	<0.001	(0.494; 82)		
P FT x G (SED; df)	NS			
P Y x FT x G (SED; df)	NS			
Linear regression				
P tolerance	NS	NS	0.019	NS
r^2, direction			0.095; (+)	
P protected yield	-	0.003	-	NS
r^2, direction		0.056; (+)		

Table 5.6. GLAI (m^2 green leaf lamina for the top 4 leaf layers per m^2) at ear emergence in the field experiments 2012 and 2014. P-values below 0.1 are reported.

Genotype	2012 untr	2012 prot	2014 untr	2014 prot
AxC 117	-	-	4.06	4.74
AxC 21	-	-	4.27	4.98
CxL 14B	5.77	7.29	4.86	5.43
CxL 3D	4.56	5.90	-	-
CxL 41F	4.14	6.14	-	-
CxL 42C	4.58	5.98	-	-
CxL 43A	5.09	6.24	5.32	5.42
CxL 48D	4.56	5.43	-	-
CxL 5H	5.21	6.66	4.90	5.47
CxL 6E	4.27	5.74	-	-
CxL 7a	4.33	5.41	3.57	4.55
CxL 8I	4.52	6.16	-	-
L14 x R 10	3.42	4.57	-	-
L14 x R 104	3.26	4.20	-	-
L14 x R 107	4.00	4.84	-	-
L14 x R 112	2.85	3.63	-	-
L14 x R 114	5.45	5.49	4.89	5.33
L14 x R 119	4.97	5.74	-	-
L14 x R 121	4.82	5.05	-	-
L14 x R 123	6.41	8.88	-	-
L14 x R 127	4.27	5.33	3.54	3.85
L14 x R 129	4.53	4.80	-	-
L14 x R 131	4.07	4.92	-	-
L14 x R 133	4.05	5.43	4.42	4.30
L14 x R 136	4.63	6.88	-	-
L14 x R 138	4.00	6.12	-	-
L14 x R 15	4.40	4.81	-	-
L14 x R 16	3.87	5.31	4.18	4.86
L14 x R 19	4.02	5.05	2.56	4.38
L14 x R 20	4.53	5.47	4.29	5.12
L14 x R 22	3.29	4.14	3.55	3.82
L14 x R 23	4.47	5.83	-	-
L14 x R 35	4.44	5.22	-	-
L14 x R 43	3.33	3.91	-	-
L14 x R 5	4.55	5.32	-	-
L14 x R 50	3.73	4.72	-	-
L14 x R 55	3.97	5.14	-	-
L14 x R 59	4.20	4.10	-	-
L14 x R 62	3.14	4.63	-	-
L14 x R 66	4.18	4.96	-	-
L14 x R 68	5.54	5.81	-	-
L14 x R 7	4.45	5.25	-	-
L14 x R 70	5.12	5.85	-	-
L14 x R 74	3.93	4.51	-	-
L14 x R 80	4.16	4.46	-	-
L14 x R 82	4.77	5.81	-	-
L14 x R 85	4.10	5.05	-	-
L14 x R 89	4.59	4.92	-	-
L14 x R 91	3.90	4.03	-	-
L14 x R 92	4.76	4.98	4.61	4.84
L8xR 10	-	-	4.79	5.09
L8xR 86	-	-	4.27	4.47
Rialto	4.47	5.46	-	-
Average	**4.36**	**5.46**	**4.26**	**4.79**
12 common genotypes	4.55	5.57	4.22	4.78
CIMMYT	4.27	5.14	4.11	4.61
UK	4.68	6.04	4.50	5.10
P Fungicide (SED; df)	0.010	(0.100;2)	0.006	(0.041; 2)
P genotype (SED; df)	<0.001	(0.246; 192)	<0.001	(0.245; 60)
P FT x G (SED; df)	<0.001	(0.349; 192)	0.075	(0.346; 60)
P FT (SED; df)	0.001	(0.329; 4)		
P G (SED; df)	<0.001	(0.316; 88)		
P Y x FT (SED; df)	0.067	(0.466; 4)		
P Y x G (SED; df)	<0.001	(0.447; 88)		
P FT x G (SED; df)	0.008	(0.447; 88)		
P Y x FT x G (SED; df)	0.020	(0.632; 88)		
Linear regression				
P tolerance	NS	NS	0.003	0.019
r^2, direction			0.163; (+)	0.095; (+)
P yield (each treatment)	<0.001	<0.001	<0.001	NS
r^2, direction	0.257; (+)	0.143; (+)	0.276; (+)	

The GLAI of the 12 common genotypes decreased in 2014 compared to 2012 (4.5 vs. 5.1, respectively; p=0.003), and displayed a significant fungicide effect and a near-significant Y x FT interaction (p=0.001 and p=0.067, respectively; Table 5.6). GLAI also displayed a genotype effect (p<0.001) as well as Y x G, FT x G and Y x FT x G interactions (p<0.001, p=0.008, p=0.020, respectively; Table 5.6). A significant relationship was also observed between the GLAI of protected crops in 2012 and 2014 (p<0.001, r^2 = 0.400; Fig 5.1e).

There were differences in leaf and canopy traits between the groups of genotypes, when divided into “CIMMYT lines” (L14 x Rialto and L8 x Rialto DH lines) and “UK-derived lines” (Rialto, Cadenza x Lynx DH lines, Avalon x Cadenza DH lines), in 2012 and 2014 (Fig 5.2). The CIMMYT lines had wider flag leaves in both 2012 and 2014 compared to the UK lines (p<0.001 and p=0.006, respectively; Table 5.3). On the other hand, the UK lines had generally longer flag-leaves, significantly so in 2014 when the UK lines were on average 1.2 cm longer than the CIMMYT genotypes (p=0.006; table 5.2). The CIMMYT lines did, however, have a slightly larger flag-leaf area in 2012 (39.2 cm^2 vs. 37.6 cm^2, p=0.05) but not in 2014. There was no difference in LAI between the groups in either year. In 2012, the UK lines having higher GLAI than the CIMMYT lines in both protected and untreated plots (p=0.006 and p<0.001, respectively) (Table 5.6). In 2014, GLAI was higher in the UK group in the fungicide-protected plots (p=0.007) but not in the untreated (p=0.094). In addition, it is important to note that the CIMMYT lines had larger ranges in all traits compared to the UK lines (Fig 5.2).

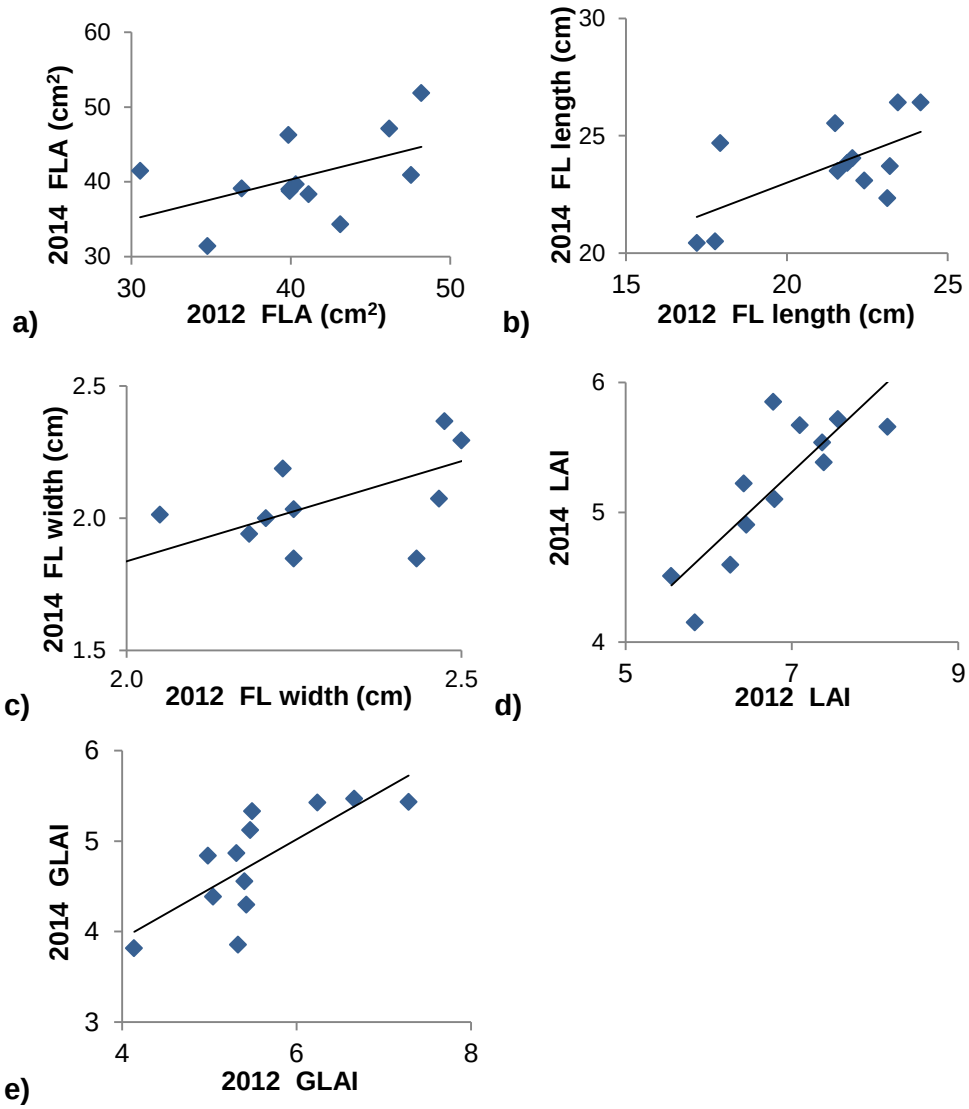
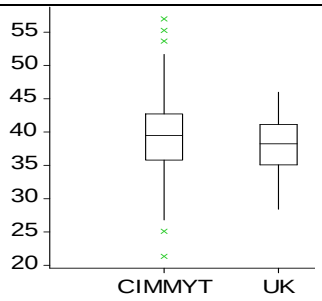
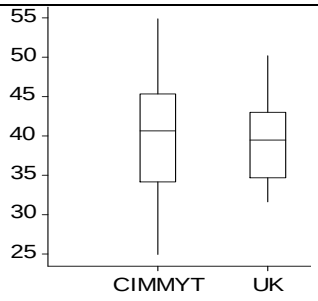
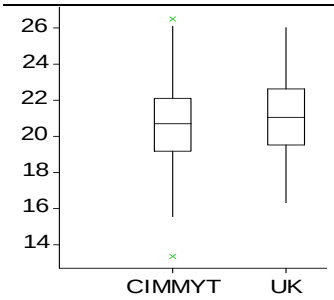
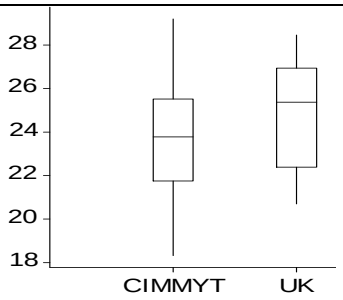
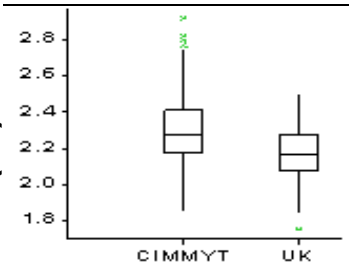
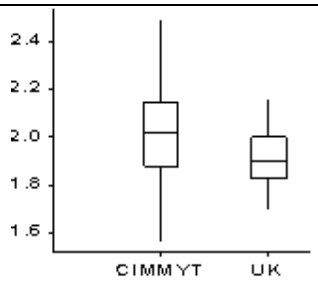
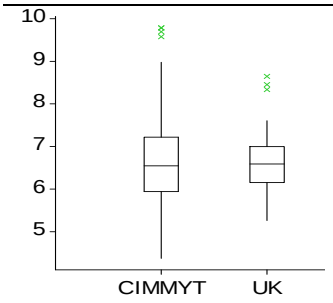
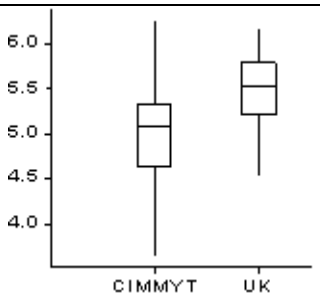
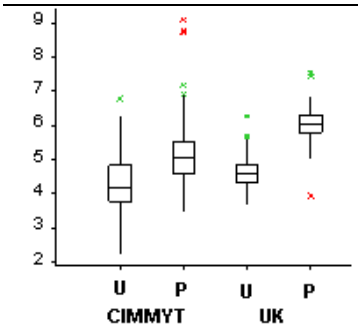
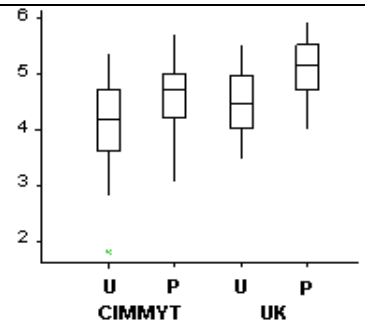


Fig 5.1. The relationship between leaf traits of the 12 common genotypes in the field experiments 2012 and 2014. a) Total flag leaf area (cm^2; p=0.011, r^2=0.077); b) Flag leaf length (cm; p=0.002, r^2=0.121); c) Flag leaf width (cm, p<0.001, r^2=0.269); d) Leaf area index (m^2 m^{-2}; p<0.001, r^2=0.349) e) Green leaf area index in the fungicide protected treatment (m^2 m^{-2}; p<0.001, r^2=0.400).

Fig 5.2. Differences between "CIMMYT genotypes" and "UK genotypes" in leaf traits during field seasons 2012 and 2014. Differences between groups were analysed using a one-way ANOVA with blocks. Reported are significant ($p<0.05$) and near-significant ($p<0.20$) values, degrees of freedom = 2 for all analyses. Outliers, more than 1.5 inter-quartile ranges from the upper quartile, are displayed in green while far outliers (more than 3 interquartile ranges from the upper quartile) are displayed in red.

	2012	2014	Probabilities
Flag leaf area (cm^2)			**2012** p = 0.050 SED = 0.751 **2014** p = NS SED = 1.370
Flag leaf length (cm)			**2012** p = 0.166 SED = 0.317 **2014** p = 0.022 SED 0.534
Flag leaf width (cm)			**2012** p <0.001 SED = 0.0263 **2014** p = 0.006 SED = 0.0404
LAI (leaf lamina m^{-2})			**2012** p = 0.173 SED = 0.136 **2014** p = NS SED = 2.85
GLAI (Green leaf lamina m^{-2})			**2012** P untr = 0.006 SED = 0.148 P prot. <0.001 SED = 0.180 **2014** P untr = 0.094 SED = 0.226 P prot. = 0.007 SED = 0.751

5.3.2. PAR interception and extinction coefficient

Fractional radiation interception (RI) was determined using a measurement of PAR at GS59 and GS73 in 2014. There was a trend for a slightly higher RI in the protected plots of 0.94 compared to 0.92 in the untreated plots, at ear emergence ($p=0.094$). There was no FT x G interaction, but there were significant genotype differences ($p<0.001$). The overall RI at GS59 was 0.93, ranging from 0.91 – 0.96 (CxL43A - CxL5H; $p<0.001$; Table 5.7). There was a positive relationship between RI and disease tolerance in the untreated treatment accounting for 17.3% ($p=0.002$), but no relationship in the protected treatments at this assessment. There was also a positive relationship between genetic variation in RI and yield in both the protected ($p=0.001$, $r^2=0.193$; Table 5.7) and the untreated ($p<0.001$; $r^2=0.396$) treatment. In addition, there were also linear relationships between RI at ear emergence and biomass at ear emergence ($p=0.021$, $r^2=0.046$) and between RI and physiological maturity ($p=0.011$, $r^2=0.057$). There was no significant difference between the UK and CIMMYT-L14/8 derived groups at GS59, but a trend was observed that suggested the RI was slightly higher in the UK lines (0.94) compared to CIMMYT lines (0.93; $p=0.099$). In addition, the RI range was slightly larger in the UK-derived lines (0.91 to 0.96) than in the CIMMYT L14/8 genotypes (0.91 to 0.95).

At GS73, fungicide-protected RI (0.92) was higher than the untreated (0.88; $p=0.023$). There was a FT x G interaction ($p=0.008$) and genotype differences ($p<0.001$) ranging from 0.83 – 0.93 (L14xR22 – CxL5H) in untreated and 0.89 – 0.96 (L14xR127 – CxL5H) in fungicide-treated conditions. There was a weak relationship between protected RI at GS73 and disease tolerance ($p=0.046$; $r^2=0.064$), and a stronger relationship between untreated RI and disease tolerance accounting for 32.7% ($p<0.001$; Table 5.7). There was also a positive linear relationship between protected RI and protected yield ($p<0.001$, $r^2=0.239$; Table 5.7), and a strong relationship between untreated grain yield and untreated RI accounting for 50.0% of variation ($p<0.001$). In addition, there was also relationship between biomass at harvest and RI at GS71 ($p<0.001$, $r^2=0.179$). There was no difference between the CIMMYT and UK genotypes in untreated or fungicide-protected conditions at GS73; however, the genotypes that had the most (L8xR10, L8xR86) and least (L14xR19, L14xR22) fractional PAR interception between fungicide treatments all belonged to the CIMMYT group.

The light extinction coefficient (k_{PAR}) was calculated from GLAI and fractional light interception at GS59. There were no FT effects or FT x G interaction. The average k_{PAR} was 0.61, and genotypes ranged from 0.44 to 0.72 ($p<0.001$). There was no relationship between k_{PAR} and disease tolerance or between k_{PAR} and protected yield (Table 5.7).

Table 5.7. Fractional PAR interception (at GS59 and GS73) and light extinction coefficient measured at GS59 (k_{PAR}) in the 2014 field experiment. Reported are significant ($p<0.05$) and near-significant ($p<0.10$) values.

	GS59		**GS59**	**GS73**	
	Fractional interception		k_{PAR}	Fractional interception	
Genotype	Untreated	Protected		Untreated	Protected
AxC 117	0.910	0.938	0.59	0.865	0.906
AxC 21	0.925	0.932	0.59	0.868	0.904
CxL 14B	0.953	0.967	0.63	0.911	0.953
CxL 43A	0.892	0.920	0.44	0.857	0.901
CxL 5H	0.958	0.966	0.64	0.927	0.957
CxL 7A	0.922	0.949	0.69	0.866	0.938
L14xR 114	0.920	0.925	0.51	0.867	0.897
L14xR 127	0.899	0.925	0.67	0.843	0.890
L14xR 133	0.924	0.938	0.62	0.906	0.933
L14xR 16	0.925	0.950	0.63	0.896	0.938
L14xR 19	0.901	0.940	0.80	0.831	0.897
L14xR 20	0.922	0.945	0.59	0.901	0.935
L14xR 22	0.895	0.924	0.63	0.830	0.891
L14xR 92	0.920	0.946	0.58	0.884	0.916
L8xR 10	0.943	0.956	0.61	0.922	0.944
L8xR 86	0.921	0.946	0.63	0.908	0.932
Average	**0.921**	**0.942**	**0.62**	**0.880**	**0.921**
CIMMYT	0.917	0.940	0.626	0.879	0.917
UK	0.927	0.945	0.596	0.882	0.927
ANOVA					
P fungicide	0.094		NS	0.023	
SED (df)				0.00633 (2)	
P genotype	<0.001		<0.001	<0.001	
SED (df)	0.00582 (60)		0.0366 (60)	0.00656 (60)	
P FT x G	NS		NS	0.006	
SED (df)				0.00927 (60)	
Linear regression					
P tolerance	0.002	NS	NS	<0.001	0.0046
r^2; direction	0.173 (+)			32.7%	0.064 (+)
P protected yield		0.001	NS		<0.001
r^2; direction		0.193 (+)			0.239 (+)

5.3.3 Radiation-Use Efficiency

The radiation-use efficiency of the 16 genotypes in 2014 was determined for the pre-anthesis (GS32 to GS63) and post-anthesis (GS63 – harvest) periods. The average pre-anthesis RUE was 3.27 g MJ PAR^{-1}, but there were no significant differences between genotypes (ranging 2.87 – 3.74 g MJ PAR^{-1}; Table 5.8) or between the CIMMYT and UK groups. Similarly, there were no differences between fungicide treatments or a FT x G interaction. There was no relationship between pre-anthesis RUE and protected yield or disease tolerance. There was a strong relationship between genetic variation in pre-anthesis RUE and biomass at anthesis ($p<0.001$, $r^2= 0.753$; Fig 5.3b), but as the RUE values were calculated using the biomass data there is a possible auto-correlation and should thus be interpreted cautiously.

In contrast to the pre-anthesis period, differences in RUE were observed between fungicide treatments in the post-anthesis period ($p=0.004$), where the average untreated RUE was 3.23 g MJ PAR^{-1} compared to 2.65 g MJ PAR^{-1} when fungicide protected (Table 5.8). There was no FT x G interaction, but there were large genotype ranges; in untreated conditions they ranged between 1.42 – 5.08 g MJ PAR^{-1} (L14xR19 – CxL43A) and in fungicide-protected conditions 1.52 – 3.86 g MJ PAR^{-1} (L14xR19 – CxL43A; $p<0.001$). There was no relationship between post-anthesis RUE and disease tolerance or protected yield, and no differences between UK and CIMMYT lines.

Table 5.8. Radiation-use efficiency for pre-anthesis (GS32-GS63) and post-anthesis (GS63 – physiological maturity) periods for 16 genotypes in the 2014 field experiment. Reported are significant ($p<0.05$) and near-significant ($p<0.10$) values.

	Pre-anthesis RUE (g MJ PAR^{-1})		Post-anthesis RUE (g MJ PAR^{-1})	
Genotype	Untreated	Protected	Untreated	Protected
AxC 117	2.77	3.15	3.39	2.45
AxC 21	3.06	3.09	2.73	1.90
CxL 14B	2.84	3.02	3.50	2.68
CxL 43A	3.14	3.09	5.08	3.86
CxL 5H	3.23	3.42	3.26	3.26
CxL 7A	2.94	3.35	2.17	2.12
L14xR 114	3.11	3.27	4.31	3.71
L14xR 127	3.78	3.71	2.58	1.93
L14xR 133	3.38	3.80	4.22	2.90
L14xR 16	3.37	3.18	3.50	2.70
L14xR 19	3.20	3.53	1.55	1.52
L14xR 20	3.12	3.26	3.02	2.14
L14xR 22	3.54	3.40	3.04	2.64
L14xR 92	3.64	3.30	3.16	2.74
L8xR 10	3.20	2.54	3.23	3.34
L8xR 86	3.55	3.60	3.09	2.46
Average	**3.24**	**3.29**	**3.28**	**2.65**
CIMMYT	3.40	3.36	3.23	2.61
UK	3.00	3.19	3.36	2.71
ANOVA				
P fungicide	NS		0.004	
SED (df)			0.037 (2)	
P genotype	NS		<0.001	
SED (df)			0.537 (59)	
P FT x G	NS		NS	
SED (df)				
Regression				
P tolerance r^2; direction	NS	NS	NS	NS
P protected yield r^2; direction		NS		NS

5.3.4 Crop growth rate

The crop growth rates of each genotype were determined for the pre-anthesis (GS32 – GS63) and post-anthesis period (GS63 – physiological maturity) in 2014. The mean pre-anthesis growth rate was 19.25 g m^{-2} day^{-1} (genotype range 16.9 – 22.1 g m^{-2} day^{-1}), but there was no significant differences between fungicide treatments, genotypes and no FT x G interaction (Table 5.9). There was a slight decrease in post-anthesis crop growth rate at 15.3 g m^{-2} day^{-1}, with significant genotype differences from 9.48 to 23.16 g m^{-2} day^{-1} (L14x127 - CxL43A; $p<0.001$). There were no differences between fungicide treatments or FT x G interaction. There was no relationship between pre-anthesis crop growth rate and disease tolerance but there was an association between disease tolerance and decreased post-anthesis crop

growth rate (p=0.001; r^2=0.099). There were no relationships with protected grain yield in either pre-anthesis or post-anthesis growth rates.

Table 5.9. Crops growth rates pre- and post-anthesis for the 16 genotypes studied in the 2014 field experiment. Reported are significant (p<0.05) and near-significant (p<0.1) values.

Genotype	Crop growth rate pre-anthesis (g m^{-2} day^{-1})	Crop growth rate post-anthesis (g m^{-2} day^{-1})
AxC 117	16.9	15.4
AxC 21	17.6	12.7
CxL 14B	18.4	18.0
CxL 43A	17.3	23.2
CxL 5H	21.1	18.1
CxL 7A	19.2	10.7
L14xR 114	18.3	20.9
L14xR 127	22.1	9.5
L14xR 133	21.5	18.8
L14xR 16	19.7	16.4
L14xR 19	17.0	8.6
L14xR 20	19.0	13.9
L14xR 22	20.3	11.2
L14xR 92	20.3	15.5
L8xR 10	18.0	18.3
L8xR 86	21.7	14.0
Average	**19.3**	**15.3**
Protected avg.	19.9	15.3
Untreated avg.	18.6	15.3
CIMMYT	19.8	14.7
UK	18.4	16.3
ANOVA		
P fungicide	NS	NS
SED (df)		
P genotype	NS	<0.001
SED (df)		2.73 (60)
P FT x G	NS	NS
SED (df)		
Linear regression		
P tolerance	NS	0.001
r^2, direction		0.099; (-)
P protected yield	NS	NS
r^2, direction		

5.3.5 Biomass and dry matter partitioning

Biomass measurements were performed at the start of stem extension (GS32), early flowering (GS63) and at physiological maturity (physiological maturity) in 2014.

5.3.5.1 GS32

The above-ground biomass at GS32 averaged 104.2 g m^{-2}, with genotypes ranging between 60.9 and 148.1 m^{-2} (p<0.001; Table 5.10). Overall the CIMMYT lines had higher biomass at GS32 compared to the UK genotypes (110.3 vs. 93.9 g; p=0.030), but there was no fungicide effect or FT

x G interactions. There was a positive relationship between biomass at GS32 and untreated yield (p=0.006, r^2=0.134), but no associations with disease tolerance or protected yield.

5.3.5.2 GS63

At GS63, overall biomass had increased to 970.6 g m^{-2}, with a genotype range from 843.6 to 1106.0 g m^{-2} (p=0.065). Again the CIMMYT lines had higher biomass than the UK genotypes (999.7 vs. 922.1 g m^{-2}; p=0.039). There were no fungicide effects or FT x G interactions at this sampling. There were no relationship between biomass at GS63 and disease tolerance or protected yield, but there was a positive relationship between untreated biomass and untreated yield (p=0.004, r^2=0.146).

At GS63, dry matter was partitioned into leaf lamina, stem plus leaf sheath, and ears. Stem DM ranged amongst genotypes from 536 – 784 g m^{-2} (AxC21 – L8xR86; p=0.003; Table 5.10), but there was no fungicide effect or FT x G interaction. There were also no fungicide effects or interactions when the stem DM was expressed as proportion of above-ground DM. However, there were genotype differences ranging 0.63 - 0.72 (p<0.001; Fig 5.5). There was a trend for the CIMMYT lines to have a higher stem DM than the UK lines (657.9 g vs. 611.6 g m^{-2}, p=0.085), but there was no difference between the groups in stem DM partitioning.

There was no relationship between stem dry weight and disease tolerance when expressed as dry weight per m^{-2} and but a weak negative trend when expresses as a proportion of above-ground DM (p=0.059, r^2=0.027). There were no relationships between stem dry weight (expressed either as DM or proportion) and protected yield (Table 5.10).

Table 5.10. Above-ground biomass at growth stages 32, 63 and the stem, leaf and ear partitioning at GS63. Reported are significant (p<0.05) and near-significant (p<0.10) values. The values are averaged over both fungicide treatments unless stated otherwise.

Genotype	GS32 DM (g m^{-2})	GS63 DM (g m^{-2})	GS63 stem DM (g m^{-2})	GS63 Leaf DM (g m^{-2})	GS63 Ear DM (g m^{-2})
AxC 117	83.6	843.6	560.7	152.6	130.3
AxC 21	60.9	852.5	535.9	168.2	148.5
CxL 14B	132.1	961.9	652.5	170.7	138.7
CxL 43A	85.0	862.6	579.7	152.0	131.0
CxL 5H	108.2	1056.3	735.5	164.1	156.8
CxL 7A	93.9	955.6	605.5	174.1	176.1
L14xR 114	98.8	921.7	585.4	155.7	180.7
L14xR 127	101.4	1095.3	718.7	141.0	235.6
L14xR 133	99.5	1068.9	698.4	167.1	203.5
L14xR 16	113.4	1000.1	664.9	161.4	173.8
L14xR 19	93.8	857.9	631.6	127.2	223.3
L14xR 20	101.9	955.1	577.5	153.3	224.4
L14xR 22	122.7	1035.1	684.5	143.0	207.6
L14xR 92	92.2	1004.1	644.0	156.0	204.0
L8xR 10	148.1	953.3	683.2	146.0	124.1
L8xR 86	131.5	1106.1	784.2	156.6	165.1
Average	**104.2**	**970.6**	**646.4**	**156.8**	**176.5**
Protected avg.	114.5	1011.4	663.4	167.2	180.8
Untreated avg.	93.8	929.9	629.4	146.4	172.1
CIMMYT	110.3	999.7	657.9	150.7	191.1
UK	93.9	992.1	611.6	163.6	146.9
ANOVA					
P fungicide	NS	NS	NS	0.024	NS
SED (df)				3.31 (2)	
P genotype	<0.001	0.065	0.003	NS	<0.001
SED (df)	14.27 (60)	93.46 (60)	64.24 (60)		17.33 (60)
P FT x G	NS	NS	NS	NS	NS
SED (df)					
Linear regression					
P tolerance	NS	NS	NS	NS	NS
r^2, direction					
P protected yield	NS	NS	NS	NS	NS
r^2, direction					

There was a difference between fungicide treatment on leaf lamina dry weight, with dry weights of 143.9 and 167.2 g m^{-2} for untreated and fungicide protected conditions, respectively (p=0.024). There were no genotype differences and no FT x G interactions (Table 5.10). In contrast, for leaf lamina partitioning there was no difference between fungicide treatments or FT x G interactions but a genotype difference, with a range between 0.13 – 0.20 (p<0.001; Fig 5.3). There was no relationship between leaf lamina dry weight

and disease tolerance or between leaf lamina partitioning and tolerance. On the other hand, there was no relationship between leaf lamina DM per m^2 or leaf lamina partitioning and protected yield. There was a difference in leaf lamina DW between UK and CIMMYT genotypes (163.6 vs. 153.1 g m^{-2}, respectively; p=0.045), and the UK genotypes also had higher leaf lamina partitioning (0.171 vs. 0.148, p<0.001).

There were genotypic differences in ear DM per m^{-2}, ranging between 124.1 and 235.6 g m^{-2} (L8xR10 - L14xR127; p<0.001). On average, the CIMMYT genotypes had considerably higher ear weight compared to the UK genotypes (194.0 g m^{-2} vs. 146.8 g m^{-2}, p<0.001; Table 5.10). There was no fungicide treatment effect or FT x G interaction for ear DM or partitioning (Table 5.10). The average ear partitioning at GS63 was 0.180, ranging between genotypes from 0.129 to 0.235 (p<0.001). There was no relationship between ear DM per m^{-2} and disease tolerance, but there was a weak negative trend between ear partitioning and disease tolerance (p=0.092; r^2=0.020). There was no relationship with ear dry weight or partitioning and protected grain yield.

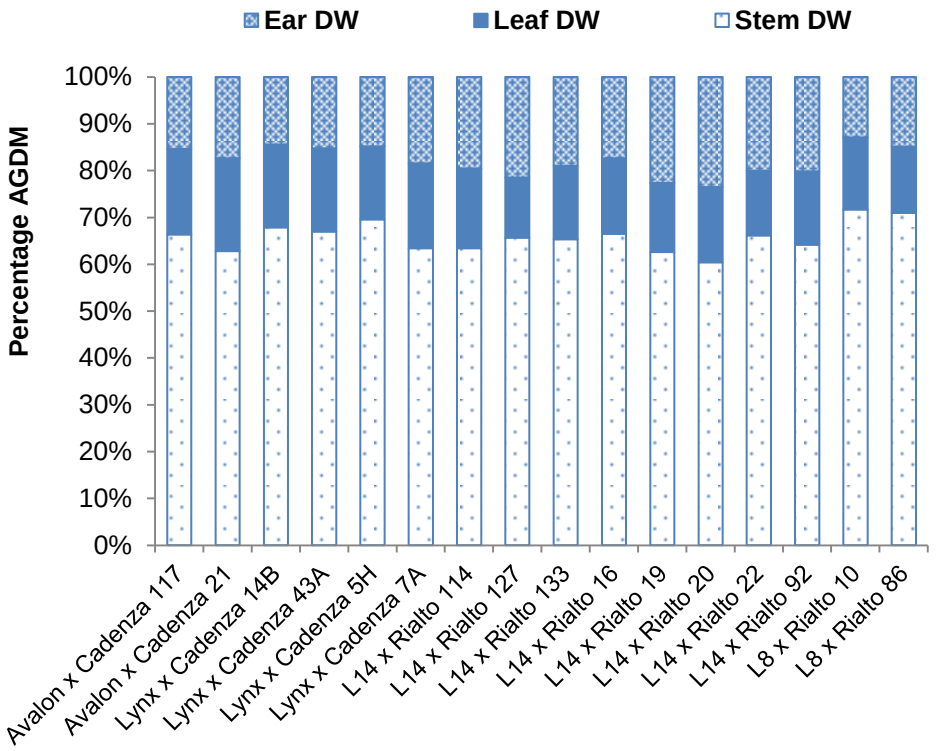


Fig 5.3. Above-ground dry matter (AGDM) partitioning into stems (incl. leaf sheaths), leaf lamina and ears for 16 genotypes at GS63 in 2014. Values represent means of fungicide-treated and untreated plots.

5.3.5.3 Harvest

There was a very weak tendency for disease to reduce biomass at harvest ($p=0.142$) but there was no FT x G interaction (Table 5.11). At physiological maturity, the average untreated biomass was 1804.8 g m^{-2}, whereas fungicide protected crops had an average biomass of 1882.6 g m^{-2}. Genotypes ranged from 1180.1 – 2190.4 g m^{-2} (L14xR19 – CxL43A) when untreated and 1512.3 – 2209.2 g m^{-2} (L14xR19 – CxL5H; $p<0.001$) when fungicide-treated. There was a positive relationship between biomass at harvest and disease tolerance accounting for 14.6% ($p<0.001$). There was no relationship with protected yield, but there was a positive relationship between biomass at harvest and untreated yield ($p<0.001$, $r^2=0.255$). There was no significant difference in biomass between the CIMMYT-derived lines and the UK genotypes.

The biomass sample at harvest was divided into ears and combined stems and leaf weights. The stem and leaf weight for individual genotypes ranged between 477.8 and 1011.3 g m^{-2} ($p<0.001$; Table 5.11). Similarly, the average stem and leaf partitioning was 0.385, ranging between 0.343 and 0.509 for individual genotypes ($p<0.001$). There was no fungicide effect or FT x G interaction for either stem and leaf weight or partitioning. There were, however, positive relationships between disease tolerance and both stem and leaf weight ($p<0.001$; $r^2=0.323$) and stem and leaf partitioning ($p<0.001$; $r^2=22.9$).

Ear dry weight at harvest ranged amongst genotypes from 868.4 – 1372.8 g m^{-2} (L14xR19 - L14xR114; $p<0.001$) and proportional ear weight ranged between 0.491 – 0.657 (L8xR10 – L14xR22; $p<0.001$). There was, however, no overall difference between UK and CIMMYT genotypes in ear dry weight or proportion at physiological maturity. There was no fungicide effect on ear weight or a FT x G interaction, and also no relationship with disease tolerance. There was a trend for high ear weight to be associated with high protected yield (Table 5.11). There were no differences between fungicide treatments and no FT x G interaction in proportional ear weight. There was a negative relationship between increased proportion ear weight and disease tolerance ($p<0.001$; $r^2=0.229$), but no relationship with protected grain yield.

In 2014, untreated harvest index (HI) was 0.43 and fungicide protected 0.51 ($p=0.040$; Table 5.12). There were significant differences between genotypes; ranging from 0.32 – 0.53 (CxL43A – L14xR19) in untreated plots

and 0.41 – 0.57 (CxL43A – AxC21) when fungicide protected ($p<0.001$). There was no significant difference in untreated harvest index between CIMMYT and UK genotypes, but there was a trend for the UK lines to have a larger protected HI than the CIMMYT lines (0.53 vs. 0.49, respectively; $p=0.088$). There was no FT x G interaction, and no relationship between untreated harvest index and disease tolerance. There was, however, a negative relationship between harvest index in the fungicide protected treatment and tolerance ($p=0.001$, $r^2=0.192$; Fig 5.4).

Table 5.11. Above-ground dry matter (AGDM) and ear and straw (stems and leaves) at harvest in the 2014 field experiment. Values represent means of fungicide treated and untreated plots unless stated otherwise. Reported are significant ($p<0.05$) and near-significant ($p<0.10$) values.

Genotype	Harvest AGDM ($g\ m^{-2}$)	Harvest Stem + leaf DM ($g\ m^{-2}$)	Harvest Ear DM ($g\ m^{-2}$)
AxC 117	1720.3	596.6	1123.6
AxC 21	1574.4	603.1	971.3
CxL 14B	1989.7	827.5	1162.2
CxL 43A	2183	848.9	1334.2
CxL 5H	2086.3	860.9	1225.5
CxL 7A	1563	567.8	995.2
L14xR 114	2114.1	741.3	1372.8
L14xR 127	1635.8	567.9	1067.9
L14xR 133	2142.5	834.8	1307.8
L14xR 16	1936.5	764.5	1171.9
L14xR 19	1346.2	477.8	868.4
L14xR 20	1749	599.6	1149.4
L14xR 22	1674.7	598.2	1076.5
L14xR 92	1888.5	664.3	1224.2
L8xR 10	1994.1	1011.3	982.8
L8xR 86	1901.1	836.2	1064.9
Average	**1843.7**	**712.5**	**1131.2**
Protected	1882.6	719.8	1162.7
Untreated	1804.8	705.2	1099.6
CIMMYT	1838.2	709.6	1128.7
UK	1852.8	717.5	1135.3
ANOVA			
P fungicide	NS	NS	NS
SED (df)			
P genotype	<0.001	<0.001	<0.001
SED (df)	114.47 (60)	36.67 (60)	84.53 (60)
P FT x G	NS	NS	NS
SED (df)			
Linear egression			
P tolerance	<0.001	<0.001	NS
r^2, direction	0.146 (+)	0.323 (+)	
P protected yield	NS	NS	0.081
r^2, direction			0.044; (+)

Table 5.12. Harvest index (untreated and fungicide protected) in the 2014 field experiment. Reported are significant (p<0.05) and near-significant (p<0.01) values.

Genotype	Untreated HI	Protected HI
AxC 117	0.402	0.570
AxC 21	0.393	0.573
CxL 14B	0.483	0.539
CxL 43A	0.322	0.408
CxL 5H	0.476	0.514
CxL 7A	0.435	0.572
L14xR 114	0.378	0.426
L14xR 127	0.435	0.539
L14xR 133	0.413	0.421
L14xR 16	0.418	0.541
L14xR 19	0.526	0.570
L14xR 20	0.449	0.525
L14xR 22	0.408	0.505
L14xR 92	0.457	0.504
L8xR 10	0.401	0.395
L8xR 86	0.430	0.472
Average	**0.427**	**0.505**
CIMMYT	0.432	0.490
UK	0.418	0.529
P fungicide	0.040	
SED (df)	0.0160 (2)	
P genotype	<0.001	
SED (df)	0.0347 (60)	
P FT x G	NS	
SED (df)	0.491 (60)	
Linear regression		
P tolerance	Ns	0.001
r^2, direction		0.192; (-)
P protected yield	0.049	<0.001
r^2, direction	0.063; (+)	0.278; (+)

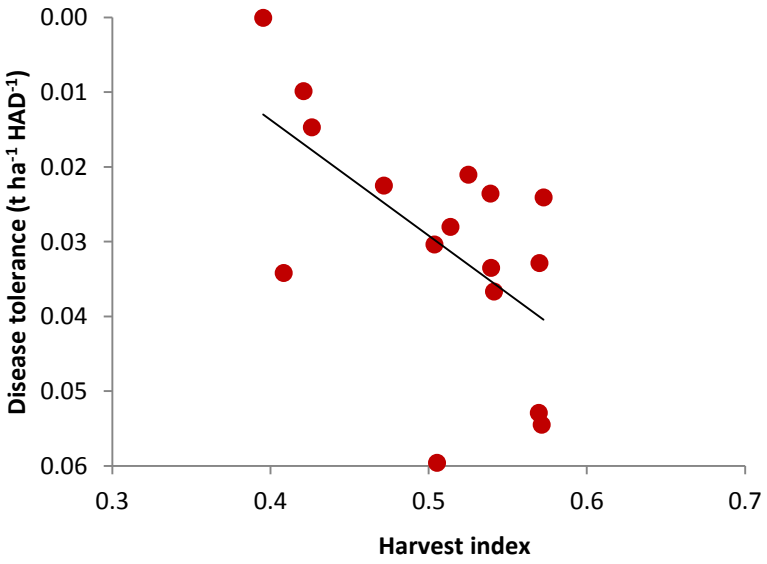
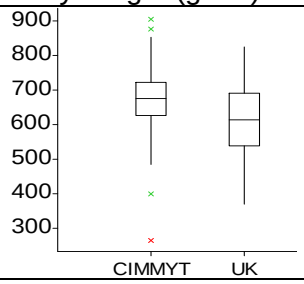
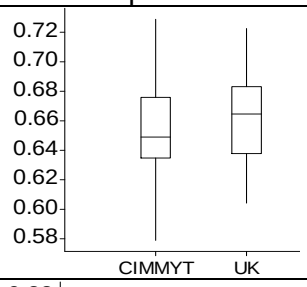
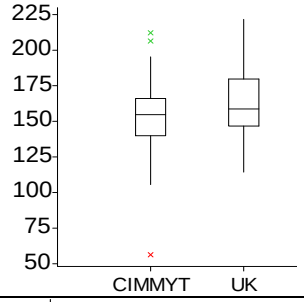
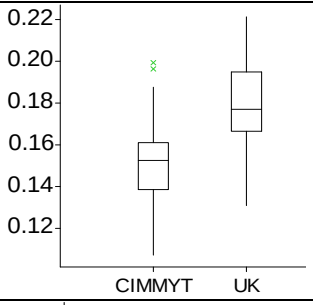
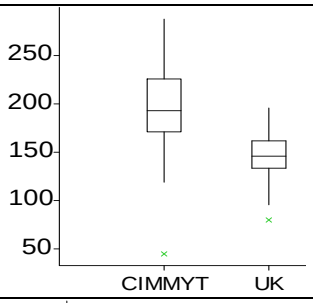
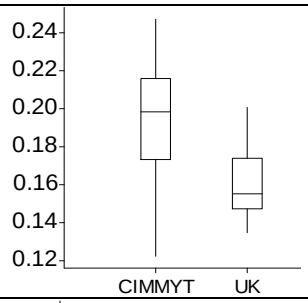
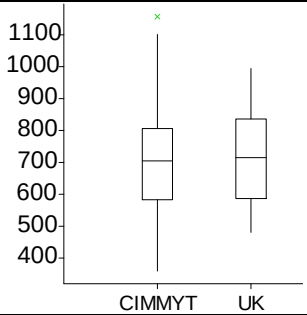
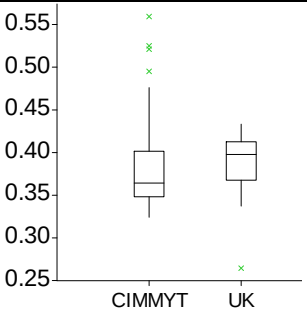
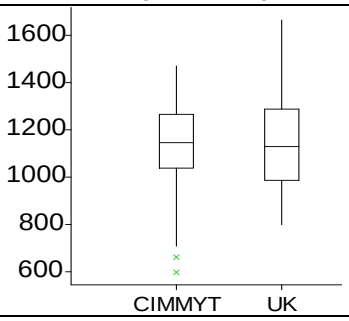
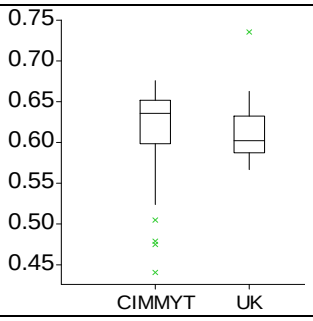


Fig 5.4. Relationship between disease tolerance (t ha^{-1} HAD^{-1}) and harvest index in fungicide protected plots for the 16 genotypes in the 2014 field experiment (p=0.001, r^2=0.192).

Figure 5.5. Differences between "CIMMYT genotypes" and "UK genotypes" in dry matter partitioning at GS63 and harvest (Har), expressed as dry weight per m^2 or proportion of total biomass. Reported are significant ($p<0.05$) and near-significant ($p<0.10$) differences between groups. Degrees of freedom = 2 for all analyses below. Outliers, more than 1.5 Inter quartile ranges from the upper quartile, are displayed in green while far outliers (more than 3 interquartile ranges from the upper quartile) are displayed in red.

	Dry weight (g m^{-2})	Proportion	Differences
GS63 Stems			P DM = 0.085 SED = 26.58 P prop. = NS SED =0.00696
GS63 Leaf lamina			P DM = 0.034 SED = 5.97 P prop. <0.001 SED = 0.00389
GS63 Ears			P DM <0.001 SED = 8.42 P prop. <0.001 SED = 0.00594
Har stems + leaves			P DM = NS SED = 33.10 P prop. = NS SED = 0.0100
Har Ears			P DM = NS SED = 41.01 P prop. = NS SED = 0.0100

5.3.6 Stem storage of water soluble carbohydrates

The amount of stem water soluble carbohydrate was determined at 7 days post ear emergence and at physiological maturity in 2014. The average stem WSC concentration at GS59 + 7d was 17.75% and amount was 150.5 g m^{-2} (Table 5.13). There were no differences between fungicide treatments and no FT x G interaction, but genotypes ranged from 9.4 - 26.0% (79.34 – 224.50 g m^{-2}; $p<0.001$). The CIMMYT genotypes had an average of 37.7 g m^{-2} higher accumulated WSC at GS59+7d compared to CIMMYT genotypes when untreated ($p=0.009$), but there was no significant difference when protected ($p=0.151$). The CIMMYT lines also had 4.2% higher accumulated WSC when untreated ($p=0.010$) and 3.2% higher WSC when fungicide protected compared to the UK lines ($p=0.051$).

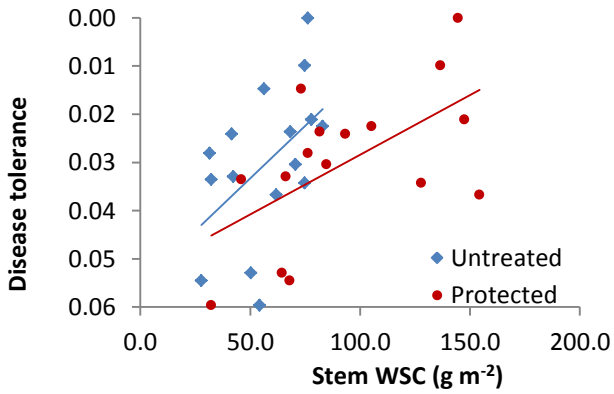
At physiological maturity, the average stem WSC concentration had decreased to 7.8% in untreated plots (57.7 g m^{-2}) and 11.5% (93.8 g m^{-2}) in fungicide-protected plots, although the difference between fungicide treatments was non-significant ($p=0.099$). There were, however, differences in stem WSC concentration and amount between genotypes ($p<0.001$), and a significant FT x G interaction ($p=0.003$; Table 5.13). There was a wide range of WSC concentration between genotypes; from 3.8 – 10.50% (CxL7A – L14xR92) in untreated plots and 5.0 – 16.7 % (L14xR22 – L14xR16) in fungicide-protected plots. There were also differences between the UK and CIMMYT genotypes when untreated (but not fungicide protected); the CIMMYT genotypes had higher WSC stem concentrations remaining at physiological maturity (8.6%) compared to the UK genotypes (6.3%; $p=0.001$), and the same was true for WSC amounts (g m^{-2}; $p=0.034$).

The amount of stem WSC utilised between GS59 + 7d and physiological maturity varied between genotypes; from an increase in WSC of 31.3 g m^{-2} at physiological maturity to a decrease of 185.4 g m^{-2} (L8xR10 - L14xR127; $p<0.001$). WSC loss was slightly higher in the untreated than the protected treatments, 79.7 g m^{-2} vs. 69.8 g m^{-2}; however, this was not statistically significant and there was no FT x G interaction (Table 5.13). There were no differences between the CIMMYT and UK genotypes in either treatment.

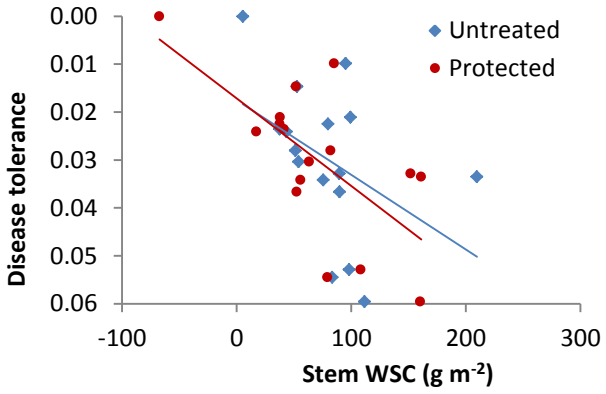
There was no relationship between stem WSC amount at GS59 + 7d or WSC utilisation and protected and untreated grain yield, but there was a

weak positive relationship between untreated physiological maturity stem WSC amount and the untreated yield (p=0.036, r^2=0.073). There was no relationship with protected grain yield at physiological maturity.

There were negative relationships between disease tolerance and stem WSC amount (p=0.027; r^2=0.041) and concentrations (p=0.003; r^2=0.078) at ear emergence. At physiological maturity however, there were positive relationships between remaining stem WSC amounts and disease tolerance in both treatments (p=0.011; r^2=0.114 and p<0.001; r^2=0.226 for untreated and protected, respectively; Fig 5.6a) and also between disease tolerance and protected WSC concentration (p=0.005; r^2=0.140; Table 5.13). There were also relationships between low WSC percentage utilisation and disease tolerance in both treatments (p=0.022; r^2=0.090 and <0.001; r^2=0.202 for untreated and protected, respectively), and for low utilisation of WSC amounts and disease tolerance (p=0.039; r^2=0.069 and p<0.001; r^2=0.218; Fig 5.6b).



a



b

Fig 5.6. The relationships between disease tolerance (t ha^{-1} HAD^{-1}) and a) untreated and protected stem WSC amounts (g m^{-2}) at physiological maturity (p=0.011, r^2=0.114 and p<0.001, r^2=0.226, respectively), and b) the WSC stem storage remobilisation between GS59+7d and physiological maturity for untreated (p=0.039, r^2=0.069) and protected fungicide treatments (p<0.001, r^2=0.218).

Table 5.13. Stem water soluble carbohydrate (WSC) concentration (%) and amount (g m^{-2}) at GS59+7 days and physiological maturity, and the difference between GS59+7d and physiological maturity for the 2014 field experiment.

Genotype	GS59 + 7d WSC (%)		WSC(g m^{-2})		Physiological maturity WSC (%)		WSC(g m^{-2})		GS59 + 7d to physiological maturity WSC (%)		WSC(g m^{-2})	
	Untr.	Prot.	Untr.	Prot.	Untr.	Prot.	Untr.	Prot.	Untr.	Prot.	Untr.	Prot.
AxC 117	16.2	19.2	148.6	172.6	6.5	8.3	50.3	64.4	9.7	10.9	98.3	108.2
AxC 21	11.8	15.4	85.0	110.3	6.0	12.6	41.5	93.3	5.8	2.8	43.5	17.0
CxL 14B	12.5	14.4	105.7	122.8	8.1	9.8	68.3	81.6	4.4	4.6	37.4	41.2
CxL 43A	15.8	17.9	150.1	183.5	8.4	13.6	74.7	127.7	7.4	4.3	75.4	55.8
CxL 5H	9.4	15.4	82.8	158.2	3.8	8.5	31.5	76.3	5.6	6.9	51.3	81.9
CxL 7A	17.0	20.4	111.0	147.0	4.9	11.3	27.8	67.9	12.1	9.1	83.2	79.1
L14xR 114	12.9	15.1	109.0	124.9	8.8	9.7	56.3	73.2	4.1	5.4	52.7	51.7
L14xR 127	26.8	25.3	242.0	207.0	5.8	7.8	32.3	45.9	21	17.5	209.7	161.1
L14xR 133	17.3	23.6	169.9	221.5	9.1	15.0	74.8	136.5	8.2	8.6	95.1	85.0
L14xR 16	19.3	23.8	151.4	206.7	7.7	16.7	61.8	154.3	11.6	7.1	89.6	52.4
L14xR 19	19.5	26.1	132.0	217.9	6.8	10.5	42.3	66.1	12.7	15.6	89.7	151.8
L14xR 20	22.6	24.5	177.0	185.3	11.8	17.8	77.9	147.5	10.8	6.7	99.1	37.8
L14xR 22	20.2	23.2	165.8	192.5	8.4	5.0	54.3	32.3	11.8	18.2	111.5	160.2
L14xR 92	14.9	16.4	124.4	148.0	10.5	11.1	70.5	84.7	4.4	5.3	53.9	63.3
L8xR 10	9.4	9.4	81.6	77.1	7.5	13.8	76.3	144.5	1.9	-4.4	5.3	-67.4
L8xR 86	16.8	15.6	162.5	142.8	10.0	12.0	82.9	105.2	6.8	3.6	79.6	37.6
Average	**16.4**	**19.1**	**137.4**	**163.6**	**7.8**	**11.5**	**57.7**	**93.8**	**8.6**	**7.6**	**79.7**	**69.8**
CIMMYT	18.0	20.3	151.6	172.4	8.6	11.9	62.9	99.0	9.3	8.4	88.6	73.3
UK	13.8	17.1	113.9	149.1	6.3	10.7	49.0	85.2	7.5	6.4	64.9	63.9
ANOVA												
P fungicide	NS		NS		0.099		0.093		NS		NS	
SED (df)					1.26 (2)		0.029 (2)					
P genotype	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
SED (df)	1.94 (60)		0.053 (60)		1.33 (60)		0.030 (60)		2.45 (60)		26.00 (60)	
P FT x G	NS		NS		0.003		0.001		NS		NS	
SED (df)					1.88 (60)		0.043 (60)					
Regression												
P tolerance	0.003		0.027		NS	0.005	0.011	<0.001	0.022	<0.001	0.039	<0.001
R^2 (dir)	0.078 (-)		0.041 (-)			0.140 (+)	0.114 (+)	0.226 (+)	0.090 (-)	0.202 (-)	0.069 (-)	0.218 (-)

5.3.7 Yield components

Grain yield, grains m^{-2}, ears m^{-2}, grains ear^{-1} and TGW were described in detail for the two years (2012 and 2014) in Chapter 4, and therefore will only be discussed in regard to their relationship to disease tolerance in this section.

Untreated grain yield was negatively related to disease tolerance in 2012 but explained only a small percentage of the variation ($p=0.002$, $r^2=0.057$). A much stronger negative relationship explaining over 20% of the variation existed between protected yield and disease tolerance ($p<0.001$, $r^2=0.203$; Fig 5.7a). In 2014, however, there was a strong positive relationship between untreated yield and disease tolerance ($p<0.001$; $r^2=0.267$) and a weaker relationship between disease tolerance and protected grain yield ($p=0.043$; $r^2=0.066$; Table 5.14; Fig 5.7b).

Higher numbers of grains m^{-2} in the protected crops were strongly associated with a reduction in disease tolerance in both 2012 ($p<0.001$, $r^2=0.144$; Fig. 5.7cb) and 2014 ($p=0.002$; $r^2=0.180$; Fig 5.7d), but not with untreated grains m^{-2} (Table 5.14). These relationships between grain number and tolerance did not appear to be mediated through ear density as there were no relationships between ears m^{-2} and disease tolerance in 2012 and only a weak relationship between protected ears m^{-2} and tolerance in 2014 ($p=0.050$; $r^2=0.061$). In contrast, there was a relationship between fungicide-protected grains ear^{-1} and disease tolerance in both 2012 ($p<0.001$, $r^2=0.131$; Fig 5.7g) and 2014 ($p=0.002$; r2=0.164; Fig 5.7h). There was no relationship between untreated grains ear^{-1} and disease tolerance in either year.

Untreated thousand grain weight displayed a positive significant relationship with disease tolerance in 2012 ($p<0.001$, $r^2=0.212$; Fig 5.7e) but there was no association with fungicide-protected TGW. In 2014 there was also a positive relationship between untreated TGW and disease tolerance ($p<0.001$, $r^2=0.245$; Fig 5.7f) and no relationship between protected TGW and disease tolerance.

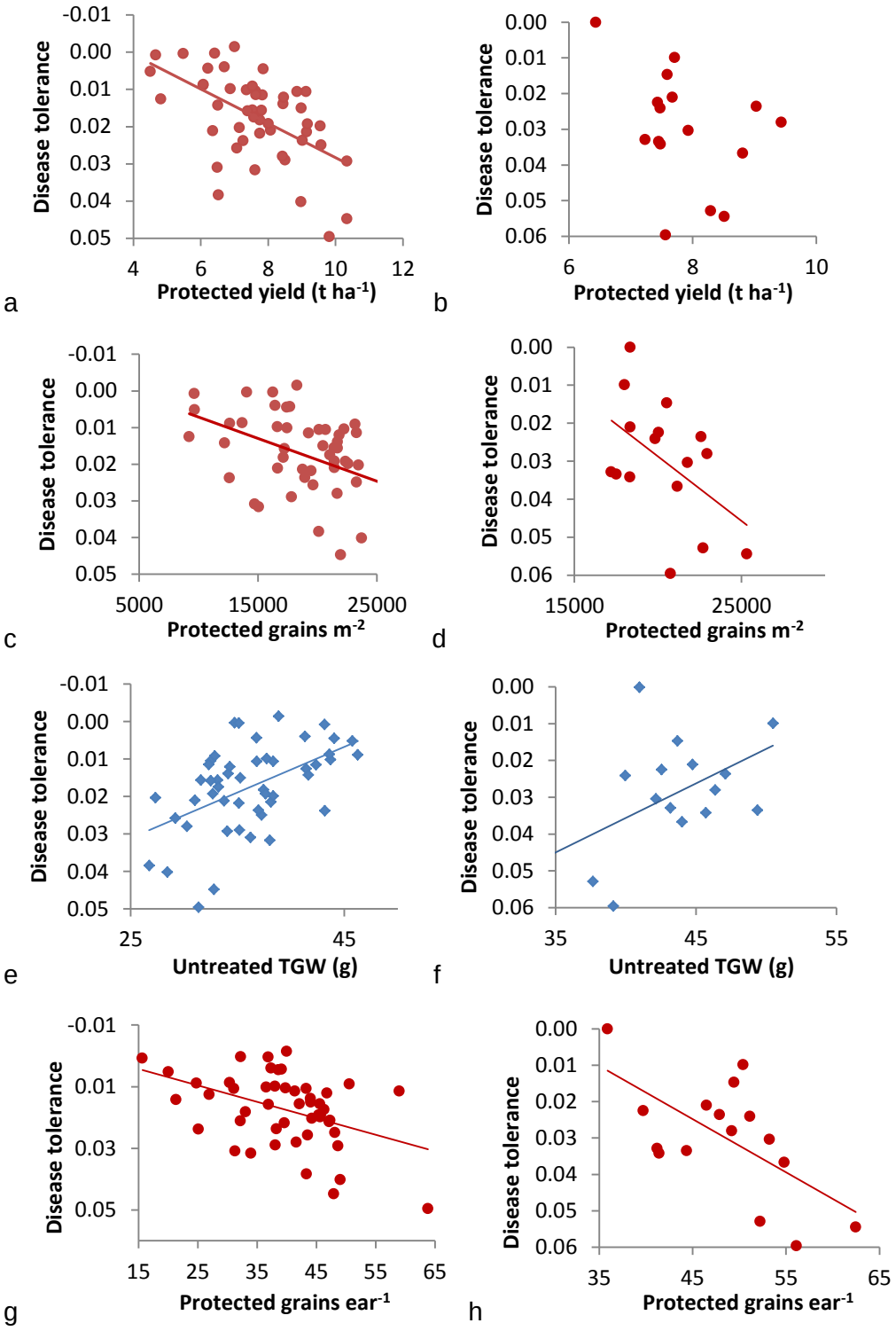


Fig 5.7. Linear regression between a) disease tolerance (t ha^{-1} HAD^{-1}) and fungicide-protected yield (85% DM) in 2012 ($p<0.001$, $r^2=0.203$), b) disease tolerance and fungicide-protected yield in 2014 ($p=0.043$, $r^2=0.066$), c) fungicide protected grains m^{-2} and disease tolerance in 2012 ($p<0.001$, $r^2=0.144$, d) fungicide protected grains m^{-2} and disease tolerance in 2014 ($p=0.002$, $r^2=0.180$), e) untreated thousand grain weight and disease tolerance in 2012 ($p<0.001$, $r^2=0.212$), f) untreated thousand grain weight and disease tolerance in 2014 ($p<0.001$, $r^2=0.245$), g) protected grains ear^{-1} and disease tolerance in 2012 ($p<0.001$, $r^2=0.131$), h) protected grains ear^{-1} and disease tolerance in 2014 ($p<0.001$, $r^2=0.164$).

Table 5.14. Summary of selected yield components, reported are significant (p<0.05) and near-significant (p<0.1) p-values.

	Grain yield (t ha^{-1}; 85% DM)		**Grains m^{-2}**		**Grains ear^{-1}**		**TGW** (g)		**Ears m^{-2}**	
	2012	2014	2012	2014	2012	2014	2012	2014	2012	2014
Averages										
Untreated	5.7	6.4	15,501	17,566	29.9	44.3	36.2	43.1	582.2	403.4
Protected	7.6	7.9	18,713	20,345	39.6	48.5	40.2	46.2	480.1	429.8
Linear regression										
P tolerance (untreated)	0.002	<0.001	ns	ns	ns	ns	<0.001	<0.001	ns	0.050
r2, direction	0.057 (+)	0.267 (+)					0.212 (+)	0.245 (+)		0.061 (+)
P tolerance (protected)	<0.001	0.043	<0.001	0.002	<0.001	0.002	ns	ns	ns	ns
r2, direction	0.203 (-)	0.066 (-)	0.144 (-)	0.180 (-)	0.131 (-)	0.164 (-)				
P protected yield	-	-	<0.001	<0.001	<0.001	<0.001	ns	0.028	ns	ns
r2, direction			0.533 (+)	0.547 (+)	0.348 (+)	0.290 (+)		0.081 (+)		
12 common genotypes										
Untreated	6.2	6.5	17,279	17,494	33.6	44.6	35.4	44	527	399
Protected	8.1	8.0	20,497	20,381	44.9	49.8	39.3	47.1	464.4	418.2
Cross year analysis										
P Fungicide treatment	<0.001		<0.001		0.014		0.005		ns	
sed; df	0.64; 4		1,625; 4		6.42; 4		2.0; 4			
P genotype	<0.001		<0.001		<0.001		<0.001		<0.001	
sed; df	0.64; 87.1		1,676; 86.4		6.02; 86.1		1.93; 87		46.71; 88	
P Y x G	<0.001		<0.001		0.066		<0.001		Ns	
sed; df	0.90; 87.1		2,370; 86.5		8.51; 86.1		2.63; 24.19			
P Y x FT	ns		ns		ns		ns		0.055	
sed; df									70.25; 4	
P FT x G	<0.001		<0.001		0.081		<0.001		ns	
sed; df	0.91; 87.1		2,401; 86.6		8.63; 86.2		2.65; 54.92			
P Y x FT x G	0.024		ns		ns		0.013		ns	
sed; df	1.28; 87.1						3.74; 51.80			

5.3.8. Source - sink balance

Healthy area duration (GLAI days) per ear and per grain was used to estimate source-sink balance in 2012 and 2014. In 2012 there was a significant G x FT interaction in HAD ear^{-1} ($p=0.009$). Most genotypes had reduced HAD ear^{-1} in the untreated compared to the protected treatment but this varied widely between genotypes; from -0.10 to 0.50 HAD ear^{-1}. Overall the untreated plots had an average HAD ear^{-1} of 0.16 and the fungicide protected HAD ear^{-1} was 0.40 ($p<0.001$; Table 5.15). The genotypes also displayed wide ranges of HAD ear^{-1} within treatments; from 0.085 – 0.31 when untreated and 0.30 – 0.51 with fungicide protection ($p<0.001$). There was a positive linear relationship between both untreated HAD ear^{-1} ($p<0.001$, $r^2=0.127$) and fungicide-protected HAD ear^{-1} ($p=0.017$, $r^2=0.032$) and disease tolerance although the latter was considerably weaker (Fig 5.8a).

In 2014 the difference between untreated and fungicide-protected HAD ear^{-1} was smaller than in 2012 at 0.227 and 0.305 respectively ($p=0.025$). In contrast to 2012 the FT x G interaction was not quite significant ($p=0.056$) but the genotype differences were ($p<0.001$; Table 5.15). The genotypes ranged from 0.121 – 0.285 and 0.215 – 0.366 HAD ear^{-1} in untreated and fungicide-protected conditions, respectively ($p<0.001$). There was a positive relationship between disease tolerance and untreated HAD ear^{-1} ($p=0.009$; $r^2=0.119$) but no relationship in the fungicide-protected treatment (Fig 5.8c).

The 12 common genotypes had a higher protected HAD ear^{-1} in 2012 of 0.39 compared to 0.31 in 2014, but higher untreated HAD ear^{-1} in 2014 than 2012 (0.22 vs. 0.17), and hence displayed Y x FT ($p=0.005$) and Y x G interactions ($p=0.018$). There was, however, no Y x FT x G interaction. The CIMMYT/Rialto lines had a higher range of HAD ear^{-1} compared to the UK genotypes in both years and for both treatments (Figure 5.10), but there were no significant differences between the groups.

There was a strong FT x G interaction in HAD $grain^{-1}$ ($p<0.001$) in 2012. Disease caused an average loss of $5.3 \cdot 10^{-3}$ HAD $grain^{-1}$, but the loss of each genotype varied from $-5.0 \cdot 10^{-4}$ to $1.8 \cdot 10^{-2}$. The overall averages were $1.09 \cdot 10^{-2}$ in the fungicide protected treatment and $5.6 \cdot 10^{-3}$ in the untreated ($p=0.004$; Table 5.16). There was a wide range of HAD $grain^{-1}$ in both treatments; the genotypes ranged $2.85 \cdot 10^{-3}$ – $1.26 \cdot 10^{-2}$ when untreated and $5.83 \cdot 10^{-3}$ – $2.39 \cdot 10^{-2}$ when fungicide protected ($p<0.001$). There was a

positive relationship between disease tolerance and untreated HAD grain^{-1} ($p<0.001$, $r^2=0.131$), and an even stronger positive relationship between tolerance and fungicide protected HAD grain^{-1} ($p<0.001$, $r^2=0.209$; Fig 5.8b).

There was also a FT x G interaction for HAD grain^{-1} in 2014 ($p=0.011$; Table 5.16). The average HAD grain^{-1} lost to disease was $1.2 \cdot 10^{-3}$, but the loss of HAD grain^{-1} ranged $-6.4 \cdot 10^{-4}$ to $3.0 \cdot 10^{-3}$ between genotypes. The overall averages were lower in the untreated plots ($5.2 \cdot 10^{-3}$) compared to the protected treatment ($6.4 \cdot 10^{-3}$), and there was a weak trend for a difference between fungicide treatments ($p=0.095$). The HAD grain^{-1} of the genotypes ranged from $3.06 \cdot 10^{-3}$ – $7.36 \cdot 10^{-3}$ and $4.15 \cdot 10^{-3}$ – $7.42 \cdot 10^{-3}$ in untreated and fungicide-protected conditions, respectively ($p<0.001$). There was a positive relationship between genetic variation in untreated HAD grain^{-1} and disease tolerance ($p=0.006$, $r^2=0.132$), and fungicide-protected HAD grain^{-1} and disease tolerance ($p<0.001$, $r^2=0.336$; Fig 5.8d).

There was no significant Y x FT x G interaction for the 12 common genotypes ($p=0.154$; Table 5.16). These genotypes displayed a similar untreated HAD grain^{-1} in 2012 as in 2014 ($5.0 \cdot 10^{-3}$ vs. $4.9 \cdot 10^{-3}$, respectively), but higher HAD grain^{-1} in 2012 ($9.0 \cdot 10^{-3}$) for the fungicide protected treatment compared to 2014 ($7.7 \cdot 10^{-3}$). Because of this, there were Y x FT and Y x G interactions ($p=0.004$ and $p<0.001$, respectively).

There was a larger range in HAD grain^{-1} in the CIMMYT genotypes than the UK lines for both treatments in 2012, but there were small differences in 2014 (Fig 5.10). The UK lines had a slightly higher untreated HAD grain^{-1} in 2014 compared to the CIMMYT lines ($p=0.046$), but the CIMMYT lines had a higher average untreated HAD grain^{-1} in 2012 ($p=0.046$). There was no difference between the two groups for the fungicide-protected HAD grain^{-1} in 2014, but the CIMMYT genotypes had considerably higher values in 2012 ($1.14 \cdot 10^{-2}$ vs. $9.10 \cdot 10^{-3}$, $p=0.003$).

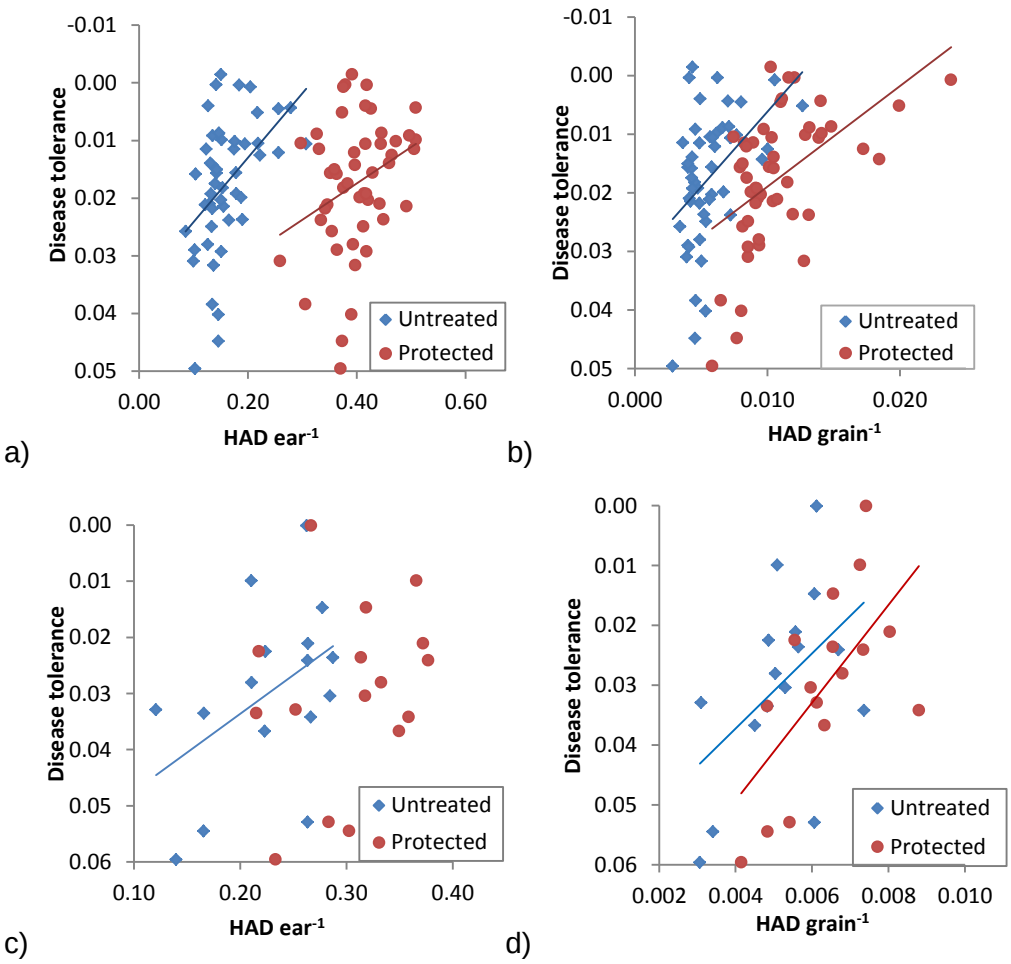


Fig 5.8. The linear relationships between a) HAD ear^{-1} and disease tolerance (t ha^{-1} HAD^{-1}) for untreated ($p<0.001$, $r^2=0.127$) and fungicide-protected treatment ($p=0.017$, $r^2=0.032$) in 2012; and b) HAD grain^{-1} and disease tolerance in the untreated ($p<0.001$, $r^2=0.132$) and fungicide-protected treatment ($p<0.001$, $r^2=0.201$) for the 49 genotypes present in the 2012 field experiment. C) HAD ear^{-1} and disease tolerance in the untreated ($p=0.009$, $r^2=0.119$) and the fungicide-protected treatment (non-significant) in 2014 D) HAD grain^{-1} and disease tolerance in the untreated ($p=0.006$, $r^2=0.132$) and fungicide-protected treatment ($p<0.001$; $r^2=0.336$) for the 16 genotypes in 2014.

Table 5.15. Summary of HAD ear^{-1} in the 2012 and 2014 field experiments.

Genotype	2012 Untreated	2012 Protected	2014 Untreated	2014 Protected
AxC 117	-	-	0.264	0.283
AxC 21	-	-	0.263	0.377
CxL 14B	0.187	0.405	0.287	0.314
CXL 3D	0.146	0.373	-	-
CxL 41F	0.137	0.397	-	-
CxL 42C	0.134	0.342	-	-
CxL 43A	0.133	0.412	0.267	0.359
CxL 48D	0.142	0.359	-	-
CxL 5H	0.189	0.449	0.211	0.333
CxL 6E	0.145	0.390	-	-
CxL 7a	0.134	0.305	0.166	0.303
CxL 8I	0.132	0.417	-	-
L14 x R 10	0.0854	0.354	-	-
L14 x R 104	0.102	0.364	-	-
L14 x R 107	0.142	0.351	-	-
L14 x R 112	0.222	0.464	-	-
L14 x R 114	0.219	0.297	0.277	0.319
L14 x R 119	0.178	0.429	-	-
L14 x R 121	0.256	0.426	-	-
L14 x R 123	0.307	0.416	-	-
L14 x R 127	0.174	0.331	0.166	0.215
L14 x R 129	0.165	0.334	-	-
L14 x R 131	0.142	0.379	-	-
L14 x R 133	0.155	0.491	0.210	0.366
L14 x R 136	0.126	0.393	-	-
L14 x R 138	0.151	0.508	-	-
L14 x R 15	0.176	0.472	-	-
L14 x R 16	0.129	0.442	0.223	0.350
L14 x R 19	0.127	0.416	0.121	0.252
L14 x R 20	0.184	0.418	0.264	0.372
L14 x R 22	0.103	0.370	0.140	0.233
L14 x R 23	0.123	0.506	-	-
L14 x R 35	0.178	0.414	-	-
L14 x R 43	0.145	0.327	-	-
L14 x R 5	0.150	0.421	-	-
L14 x R 50	0.134	0.397	-	-
L14 x R 55	0.153	0.375	-	-
L14 x R 59	0.217	0.373	-	-
L14 x R 62	0.104	0.363	-	-
L14 x R 66	0.140	0.383	-	-
L14 x R 68	0.279	0.509	-	-
L14 x R 7	0.121	0.346	-	-
L14 x R 70	0.135	0.496	-	-
L14 x R 74	0.147	0.445	-	-
L14 x R 80	0.205	0.375	-	-
L14 x R 82	0.131	0.459	-	-
L14 x R 85	0.150	0.391	-	-
L14 x R 89	0.194	0.444	-	-
L14 x R 91	0.0994	0.259	-	-
L14 x R 92	0.256	0.395	0.285	0.318
L8xR 10	-	-	0.263	0.267
L8xR 86	-	-	0.224	0.218
Rialto	0.151	0.418	-	-
Average	**0.160**	**0.400**	**0.227**	**0.305**
Common genotypes	0.166	0.394	0.218	0.311
CIMMYT	0.163	0.404	0.217	0.291
UK	0.148	0.388	0.243	0.328
P fungicide (SED; df)	<0.001	(0.0048; 2)	0.025	(0.012 ; 2)
P genotype (SED; df)	<0.001	(0.035; 192)	<0.001	(0.029; 60)
P FT x G (SED; df)	0.009	(0.049; 192)	0.056	(0.039; 60)
Cross-year ANOVA				
P FT (SED; df)	<0.001	(0.0407; 4)		
P G (SED; df)	<0.001	(0.0385; 88)		
P Y x FT (SED; df)	0.005	(0.0575; 4)		
P Y x G (SED; df)	0.018	(0.0544; 88)		
P FT x G (SED; df)	<0.001	(0.0544; 88)		
P Y x FT x G (SED; df)	NS			
Linear regression				
P tolerance	<0.001	0.017	0.009	ns
R^2, direction	0.127 (+)	0.032 (+)	0.119 (+)	
P yield (each treatment(	<0.001	NS	0.003	0.026
R^2, direction	0.075 (+)		0.154 (+)	0.083 (+)

Table 5.16. Summary of HAD grain^{-1} in the 2012 and 2014 field experiments.

Genotype	2012 Untreated	2012 Protected	2014 Untreated	2014 Protected
AxC 117	-	-	0.0061	0.0054
AxC 21	-	-	0.0067	0.0073
CxL 14B	0.0067	0.0088	0.0056	0.0065
CXL 3D	0.0045	0.0077	-	-
CxL 41F	0.0050	0.0128	-	-
CxL 42C	0.0049	0.0091	-	-
CxL 43A	0.0053	0.0085	0.0074	0.0088
CxL 48D	0.0040	0.0081	-	-
CxL 5H	0.0052	0.0119	0.0050	0.0070
CxL 6E	0.0053	0.0080	-	-
CxL 7a	0.0046	0.0065	0.0034	0.0048
CxL 8I	0.0043	0.0092	-	-
L14 x R 10	0.0034	0.0081	-	-
L14 x R 104	0.0040	0.0094	-	-
L14 x R 107	0.0040	0.0079	-	-
L14 x R 112	0.0100	0.0173	-	-
L14 x R 114	0.0057	0.0075	0.0061	0.0066
L14 x R 119	0.0058	0.0101	-	-
L14 x R 121	0.0080	0.0110	-	-
L14 x R 123	0.0072	0.0139	-	-
L14 x R 127	0.0049	0.0083	0.0048	0.0048
L14 x R 129	0.0072	0.0131	-	-
L14 x R 131	0.0041	0.0120	-	-
L14 x R 133	0.0042	0.0104	0.0051	0.0073
L14 x R 136	0.0049	0.0094	-	-
L14 x R 138	0.0061	0.0141	-	-
L14 x R 15	0.0077	0.0129	-	-
L14 x R 16	0.0041	0.0093	0.0045	0.0063
L14 x R 19	0.0049	0.0111	0.0031	0.0061
L14 x R 20	0.0062	0.0116	0.0057	0.0080
L14 x R 22	0.0028	0.0058	0.0031	0.0041
L14 x R 23	0.0036	0.0089	-	-
L14 x R 35	0.0047	0.0091	-	-
L14 x R 43	0.0066	0.0132	-	-
L14 x R 5	0.0058	0.0095	-	-
L14 x R 50	0.0096	0.0185	-	-
L14 x R 55	0.0045	0.0115	-	-
L14 x R 59	0.0126	0.0199	-	-
L14 x R 62	0.0043	0.0115	-	-
L14 x R 66	0.0043	0.0084	-	-
L14 x R 68	0.0070	0.0140	-	-
L14 x R 7	0.0056	0.0107	-	-
L14 x R 70	0.0046	0.0097	-	-
L14 x R 74	0.0071	0.0148	-	-
L14 x R 80	0.0105	0.0239	-	-
L14 x R 82	0.0043	0.0105	-	-
L14 x R 85	0.0043	0.0103	-	-
L14 x R 89	0.0059	0.0103	-	-
L14 x R 91	0.0039	0.0086	-	-
L14 x R 92	0.0060	0.0084	0.0053	0.0060
L8xR 10	-	-	0.0061	0.0074
L8xR 86	-	-	0.0049	0.0055
Rialto	0.0041	0.0085	-	-
Mean	**0.0056**	**0.0109**	**0.0052**	**0.0064**
Common	0.0051	0.0090	0.0049	0.0077
CIMMYT	0.0058	0.0114	0.0048	0.0062
UK	0.0049	0.0091	0.0057	0.0066
P fungicide (SED; df)	0.004	(0.00035; 2)	0.095	(0.00040; 2)
P genotype (SED; df)	<0.001	(0.0010; 60)	<0.001	(0.00043; 60)
P FT x G (SED; df)	<0.001	(0.0014; 60)	0.011	(0.00061; 60)
Cross-year ANOVA				
P FT (SED; df)	<0.001	(0.000802; 3.9)		
P G (SED; df)	<0.001	(0.000776; 86.1)		
P Y x FT (SED; df)	0.004	(0.001134; 4)		
P Y x G (SED; df)	<0.001	(0.001097; 86.1)		
P FT x G (SED; df)	<0.001	(0.001114; 86.1)		
P Y x FT x G (SED; df)	NS			
Linear regression				
P tolerance	<0.001	<0.001	0.006	<0.001
R^2, direction	0.132 (+)	0.201 (+)	0.132 (+)	0.336 (+)
P yield (each treatment)	0.005	<0.001	NS	NS
R^2, direction	0.046 (-)	0.369 (-)		

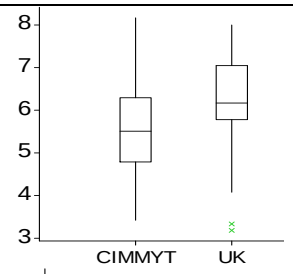
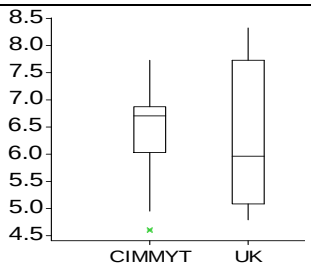
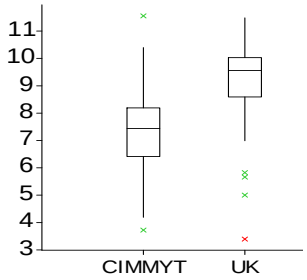
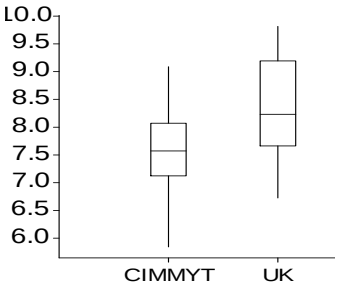
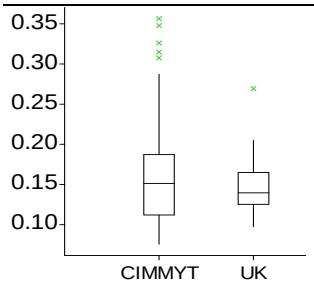
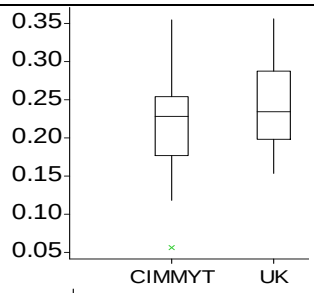
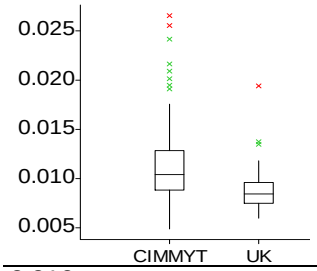
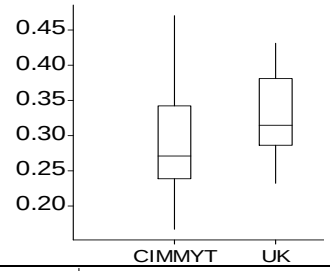
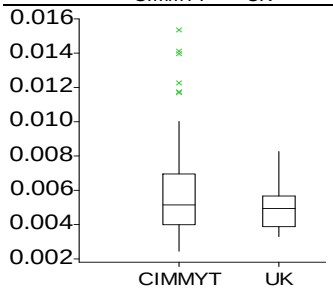
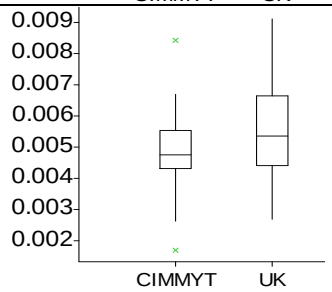
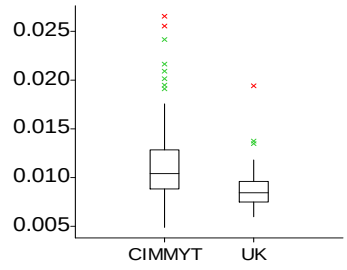
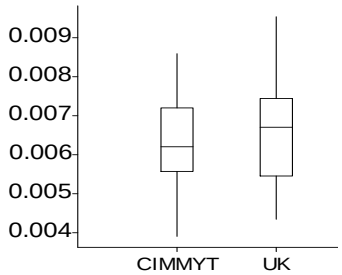
5.3.10 Ear length

Ear length was measured in 2014 as a sink size estimation, and averaged 10.47 cm and 10.60 cm in the untreated and fungicide protected treatment, respectively (Table 5.17). There was no significant fungicide effect on the ear length, but there was a strong trend for a FT x G interaction (p=0.051).The ear length in untreated conditions ranged from 9.16 – 12.26 cm (CxL14B – L14xR133), and in the fungicide-treated conditions from 9.30 – 12.63 cm (L8xR86 – L14xR133). There were no relationships between disease tolerance and ear length in either treatment or between ear length and protected yield. The CIMMYT lines had larger ear sizes than the UK genotypes in both untreated (p=0.002) and fungicide protected treatments (p<0.001).

Table 5.17. Ear length in the 2014 field experiment. Reported are significant (p<0.05) and near significant p-values (p<0.1).

Genotype	Untreated ear length (cm)	Protected ear length (cm)
AxC 117	10.92	9.71
AxC 21	9.77	10.09
CxL 14B	9.16	9.47
CxL 43A	9.53	9.93
CxL 5H	9.73	9.80
CxL 7A	10.55	10.06
L14xR 114	11.09	10.62
L14xR 127	9.87	9.99
L14xR 133	12.26	12.63
L14xR 16	10.79	11.44
L14xR 19	10.51	11.32
L14xR 20	11.51	12.63
L14xR 22	10.72	11.42
L14xR 92	10.82	10.56
L8xR 10	10.64	10.63
L8xR 86	9.71	9.30
Average	**10.47**	**10.60**
CIMMYT	10.79	11.05
UK	9.94	9.84
P fungicide (SED; df)	NS	(0.120; 2)
P genotype (SED; df)	<0.001	(0.309; 60)
P FT x G (SED; df))	0.051	(0.436; 60)
Linear regression		
P tolerance r^2, direction	NS	NS
P protected yield r^2, direction	-	NS

Fig 5.9. Differences between "CIMMYT genotypes" and "UK genotypes" in selected yield components during field seasons 2012 and 2014. Degrees of freedom = 2 in all analyses. Outliers, more than 1.5 Inter quartile ranges from the upper quartile, are displayed in green while outliers (more than 3 interquartile ranges) are in red.

Trait		2012	2014	ANOVA
Grain yield	Untreated			**2012** p = 0.13 SED = 0.219 **2014** p = ns SED = 0.312
	Protected			**2012** p <0.001 SED = 0.321 **2014** p <0.001 SED = 0.223
HAD ear^{-1}	Untreated			**2012** p = 0.157 SED = 0.0106 **2014** p = ns SED = 0.0197
	Protected			**2012** p = ns SED = 0.0168 **2014** p = 0.063 SED = 0.0195
HAD grain^{-1}	Untreated			**2012** p = 0.046 SED = 0.000442 **2014** p = 0.046 SED = 0.000413
	Protected			**2012** p = 0.003 SED = 0.000771 **2014** p = ns SED = 0.000392

5.3.11 Associations between physiological traits and disease susceptibility

Relationships between the physiological traits (measured in the fungicide protected treatment) and flag leaf STB susceptibility (when untreated) at GS59 + 14d were determined in the 2014 experiment. There were relationships between the majority of traits and disease susceptibility, and these were generally negative. There was no relationship between flag-leaf area and STB, but the other canopy traits were significantly related to flag leaf STB (Table 5.18). A higher GLAI was related to lower disease severity ($p=0.003$, $r^2=0.157$), and this was also the case for higher LAI and HAD (Table 5.18). There were also negative relationships between fractional light interception and flag-leaf disease susceptibility, particularly at GS73 (table 5.18).

There were no relationships between disease susceptibility and biomass at any sampling. There was a correlation between high disease susceptibility and lower WSC accumulation at physiological maturity ($p=0.023$; $r^2=0.089$). In contrast, a higher WSC utilisation in the absence of disease was linked to higher disease susceptibility ($p=0.006$, $r^2=0.137$). There were also significant but weak relationships between the source-sink traits and disease susceptibility; in which a high HAD grain^{-1} and HAD ear^{-1} was related to lower disease severity (Table 5.18).

Table 5.18. Relationship between selected (fungicide protected) physiological traits and flag leaf STB symptoms at GS59+14 days for the 16 genotypes present in the 2014 field experiment. P-values below 0.1 are reported.

Trait	P-value	r^2	dir.
Leaf traits			
Flag leaf area (cm^2)	NS		
Leaf lamina ($m^2\ m^{-2}$)	0.024	0.090	-
GLAI ($m^2\ m^{-2}$)	0.003	0.157	-
HAD (GLAI days)	<0.001	0.205	-
Resource capture and use			
Light interception (GS59; MJ PAR)	0.043	0.063	-
Light interception (GS73; MJ PAR)	0.003	0.147	-
K_{par}	NS		
Pre-anthesis RUE (g MJ PAR^{-1})	NS		
Post-anthesis RUE (g MJ PAR^{-1})	0.021	0.092	-
Biomass			
GS32 Biomass (g)	NS		
GS63 Biomass (g)	NS		
PM biomass (g)	NS		
Stem storage			
WSC (GS59; g m^{-2})	0.055	0.058	-
WSC (PM; g m^{-2})	0.023	0.089	-
WSC utilisation (g m^{-2})	0.006	0.137	+
Source : sink			
HAD ear^{-1} (GLAI days ear^{-1})	0.079	0.045	-
HAD $grain^{-1}$ (GLAI days $grain^{-1}$)	0.010	0.116	-
Harvest index (g g^{-1})	NS		

5.4 Discussion

There was a strong influence on disease tolerance through yield components. In 2012, disease tolerance was related to low fungicide protected yield, conferred through fewer grains m^{-2} and grains ear^{-1}. In 2014, the relationship between protected yield and disease tolerance was not statistically significant, but there were still indications that a decrease in grains m^{-2} and grains ear^{-1} was associated with increased disease tolerance. There was a relationship between genetic variation in disease tolerance and higher thousand grain weight when exposed to disease. Disease tolerance conferred through grain weight would be in agreement with previous literature on disease tolerance in wheat such as Ziv and Eyal (1976), Ziv and Eyal (1978), Ziv et al. (1981), and Zuckerman et al. (1997). Diseases such as STB restrict green area during the grain-filling period when grains m^{-2} have already been determined, so it is expected that grain weight would be decreased. However, genetic variation in TGW has been shown to have a negative relationship, with grains m^{-2} (Slafer et al., 1996). The higher TGW in the tolerant genotypes could be a consequence of lower grains m^{-2} and grains ear^{-1}, and a smaller number of grains to fill, and associated with lower protected yield. It is encouraging that there was no relationship with low protected yield in 2014 indicating that disease tolerance and low grain yield in the absence of disease is not necessarily linked (Foulkes et al., 2006, Bingham et al., 2009).

The first hypothesis was that a larger green area, both canopy and flag leaf, is beneficial to disease tolerance. This has been widely hypothesized in disease tolerance literature, in particular by Parker et al. (2004) and Bingham et al. (2009). The reasoning is that with an increased green area there will be a relatively smaller reduction in fractional radiation interception for every unit of green tissue lost, due to the exponential relationship between GAI and fractional radiation interception. Foulkes et al. (2006) found that a large flag-leaf area was conducive to disease tolerance which was further discussed by Bingham et al. (2009) and Ney et al. (2013) as a beneficial trait; a larger flag leaf would intercept a higher proportion of incoming PAR compared to lower leaves which are generally more diseased. In addition, a larger flag leaf will have a higher photosynthesizing area remaining. In this study however, there were no significant relationships indicating that flag-leaf area was related to, or

beneficial for, disease tolerance. This was also the case for the related leaf traits: flag-leaf length and width, and specific dry weight. A large green area in the form of GLAI was positively associated with disease tolerance in 2014 in both fungicide treatments, but particularly in the non-fungicide treatment, and the same was seen in untreated LAI in 2014. However, there were no relationships between disease tolerance and GLAI or LAI in 2012.

The second hypothesis was that a high proportion of light interception in the upper canopy associated with a high extinction coefficient was conducive to disease tolerance. Genotypes with more horizontal leaves in the canopy and/or leaves transmitting less radiation would in theory be less affected by disease in the lower canopy, and would also maintain a higher proportion of fractional radiation interception at a low GAI associated with green area loss due to disease (Parker et al., 2004, Paveley et al., 2008, Bingham et al., 2009). There were large differences in the extinction coefficient, kPAR, of the genotypes. These ranged between 0.44 and 0.72, which cover the most of the reported range of wheat extinction coefficient; between 0.3 and 0.7 (Acevedo et al., 2002). There have been few experimental studies on the effect of light extinction coefficient on disease tolerance, although it has been widely hypothesized as a candidate trait in combination with (or as a result of) large canopy size (Parker et al., 2004, Paveley et al., 2008, Bingham et al., 2009). A model developed by Bingham and Topp (2009) showed that an increased kPAR was related to increased disease tolerance in spring barley; however, Foulkes et al. (2006) studied kPAR in a limited range of winter wheat near-isogenic lines and did not find a relationship between the two. There was no relationship between kPAR and disease tolerance identified in this study, but there was a relationship between maintained light interception in the presence of disease and disease tolerance. It is possible that light interception itself, not conferred by kPAR, is a disease tolerance conferring trait. There was no relationship between amount of light interception and kPAR, but there were associations between untreated light interception and GLAI and LAI. These associations indicate that the increased light interception might be conferred by increased ground cover, as opposed to light extinction coefficient.

The third hypothesis stated that there would be a relationship between a lower radiation-use efficiency (RUE) and disease tolerance. The basis for this is

that loss of unit intercepted light due to disease would have a larger effect on accumulated dry matter in genotypes with high RUE (Parker et al., 2004, Paveley et al., 2008). This relationship was highlighted by Parker et al. (2004) who stated that genotypes shown to be tolerant in their study had shown lower RUE in previous literature than intolerant genotypes. The relationship between whole–season (GS32-harvest) RUE and disease tolerance was tested by Foulkes et al. (2006), who found that modern, less tolerant cultivars had higher RUE, but no direct relationship. In this study, there were no relationships between pre-anthesis or post-anthesis RUE, as expected based on previous experimental evidence. A main focus with regard to disease tolerance has been on high (or maintained) post-anthesis RUE in remaining green area in disease-tolerant genotypes - to a large extent based on the results of Zuckerman et al. (1997) who found that their intolerant genotype lost more carbon fixation capacity post-anthesis than the tolerant genotype. They also found an up-regulation in carbon fixation per unit green area in remaining green area, which has not been shown in wheat since. There was no relationship between post-anthesis RUE and disease tolerance in this study or evidence for up regulation of RUE of the remaining green area in the presence of disease.

The fourth hypothesis stated that disease-tolerant genotypes have a higher stem soluble carbohydrate storage capacity than intolerant genotypes. The rationale is that investing in larger stem storage of water soluble carbohydrates pre-anthesis and during early grain-filling which are utilized during grain filling could compensate for loss of green area caused by disease. It is an attractive theory, but currently there is no experimental evidence supporting this; in fact, Foulkes et al. (2006) found a negative correlation between disease tolerance and WSC amount. Our results support the previous findings indicating that a high amount of WSC stem storage is not conducive to disease tolerance. There was a weak relationship between decreased stem WSC at anthesis and increased tolerance. However, in this study it seems that disease tolerance is associated with a low WSC utilisation and increased WSC remaining in the stems at physiological maturity, which has not previously been shown.

A high source : sink balance in the post-anthesis period has been suggested to be important in conferring disease tolerance (Paveley et al.,

2008), and two traits representing this; HAD ear^{-1} and HAD grain^{-1} were investigated. These traits are ratios of HAD allocated to each grain (or the total grains ear^{-1}), meaning a high HAD grain^{-1} has an increased source capacity for each grain compared to a genotype with lower HAD grain^{-1}. HAD ear^{-1} and HAD grain^{-1} were positively related to disease tolerance in both years. In general, the relationships between HAD grain^{-1} and disease tolerance were slightly stronger, and particularly in the fungicide-protected treatment. Although there is no peer-reviewed experimental evidence of a relationship between HAD grain^{-1} and disease tolerance, Paveley et al. (2008) mention finding relationships between disease tolerance and both HAD grain^{-1} and green lamina area grain^{-1}. Increased HAD grain^{-1} is agreement with other traits measured in this chapter. For instance, there was an association between high leaf/stem partitioning in above-ground biomass at harvest and disease tolerance, and consequently between decreased harvest index (high biomass to grain yield ratio at harvest) and disease tolerance.

An overall aim of this project was to determine if the CIMMYT genotypes displayed an increased range of disease tolerance and physiological traits. As discussed in Chapter 4, there was an increased range in disease tolerance in the CIMMYT genotypes in both experiments and the CIMMYT genotypes displayed a generally higher disease tolerance compared to the UK genotypes. In general, these genotypes displayed increased ranges in a majority of physiological traits, in particular for source traits such as flag-leaf area, canopy size and GLAI. This supports our hypothesis that introgressing exotic germplasm into current UK-adapted germplasm is a way to find extreme contrasts in physiological traits, which can then be studied more easily.

One of the main concerns regarding disease tolerance is its apparent relationship with low protected grain yield. This relationship was displayed in 2012 but not 2014 in our study. This is encouraging, as it shows that a combination of selection for protected yield and disease tolerance may be feasible and it is possible to break the apparent link between the two. In regard to selecting physiological traits for potential use in breeding programs, potential traits should ideally not be correlated with low protected yield. As anticipated, genetic variation for yield-forming traits such as fractional light interception and harvest index displayed strong positive relationships with protected yield.

However, higher fractional light interception in the presence of disease was associated with both increased disease tolerance and increased grain yield, which is encouraging. On the other hand, harvest index displayed a negative relationship with disease tolerance. However, this is not surprising as a high harvest index is a trait bred into high-yielding modern cultivars, many of which are believed to be less tolerant than older varieties (Paveley et al., 2005). It has been suggested, as harvest indices of modern wheat cultivars are reaching their theoretical upper limit of ca. 0.62,, that future yield increases might rely on increased biomass. Because of this, it is encouraging that there was a positive relationship between disease tolerance and above-ground biomass at harvest in this study.

Disease severity was correlated with some of the physiological traits studied in 2014, in particularly for source-sink traits. Most of the yield components performed better with less disease presence, as did source traits such as GLAI and HAD. There were, however, indications of larger canopy sizes being related to less flag-leaf disease. The larger canopy size was not directly associated with the crop heights measured in Chapter 4, but might still reflect a type of disease escape effect. There was no relationship between pre-anthesis radiation-use efficiency and disease severity. Genotypes with little disease at the flag-leaf level had higher yields (and individual yield components), higher HAD, GLAI and light interception. As there was no relationship between flag-leaf disease and disease tolerance, the effects of disease resistance and escape are probably not coupled with tolerance.

In addition to STB, there were several other diseases present in the field experiments (described in Chapter 4). In the 2014 field experiment, there was yellow rust present early in the season with one genotype showing high susceptibility (L14xR19). At GS59, the average yellow rust severity on the flag leaves ranged between 0 and 2.5% for the other 11 genotypes while L14xR19 had approximately 20% yellow rust on the flag leaf. Although there is likely to be additive effects of different diseases on plant fitness traits such as yield and green leaf duration, as disease tolerance is calculated from the reduction of green leaf area it is likely to be less sensitive to the presence of small amounts of non-target diseases compared to calculations based on AUDPC or percentage target disease. It is unlikely that adding a yellow rust covariate to

the analyses would have made a large difference in the outcome, however, L14xR19 was quite badly affected by yellow rust which accounted for nearly all the reduction in HAD (88%) and consequently grain yield (85% reduction). It is possible that removing this genotype entirely from the trait analyses of this chapter would have removed some of the variation in the disease tolerance – physiological traits relationships, particularly as this genotype performed poorly throughout the season in most of the physiological traits measured.

5.5 Conclusions

Determining which traits confer disease tolerance has proven elusive for many years. In this study we have found several relationships between phenotypic traits and disease tolerance which will be of interest for future studies. The strongest relationships were between disease tolerance and source-sink balance traits such as HAD ear^{-1} and HAD grain^{-1}. There were also indications that high fractional light interception and high GLAI could benefit disease tolerance. However, none of these traits have strong enough effects to be solely responsible for displays of disease tolerance. Interestingly, none of the hypotheses regarding disease tolerance traits were entirely confirmed in this study, although there seem to be an association between larger canopy size and disease tolerance as suggested in the first hypothesis. Some of the results were in agreement with previous studies, for instance the lack of influence of RUE on disease tolerance, while others were not. These results should however be interpreted cautiously as most of the phenotype traits are from a single field experiment using a limited number of genotypes; this study would benefit from several years of further field experiments.

6. Quantifying the relationships between disease severity and pathogen load in controlled environments

6.1 Introduction

In order to conduct plant pathology studies, reliable quantification of disease is essential. Visual assessments of disease symptoms are generally performed in order to quantify disease presence, commonly as a percentage of the total leaf area infected. However visual disease scores are subjective, relying on the consistency of the assessor (Cooke, 2006), and also time consuming and reliant on adequate manpower. A promising alternative is the quantification of pathogen levels through DNA using Real Time quantitative PCR (qPCR). Pathogen detection using qPCR has been demonstrated for a range of starting materials, including soil, roots, grains and leaves (Bearchell et al., 2005, Guo et al., 2006, Nicolaisen et al., 2009, Wallenhammar et al., 2012). In addition, it is particularly well suited for the detection of hemibiotrophic pathogens, which do not display visible symptoms until well established within the host. This method has been shown to detect pre-symptomatic disease and could be a useful tool in disease predictions and fungicide spray decisions (Schena et al., 2004, Guo et al., 2006). In the literature, although general guidelines have been published for reporting qPCR (Bustin et al., 2009), different methods are used to express the detected pathogen DNA. Most studies standardise pathogen DNA by total extracted DNA (Fraaije et al., 2001, Burlakoti et al., 2007, Nicolaisen et al., 2009), others by wet or dry sample weights (Reischer et al., 2004, Fredlund et al., 2008), while some do not report which method was used for standardisation (Bates et al., 2001, Guo et al., 2006).

Zymoseptoria tritici (the causal agent of Septoria tritici blotch) is the most important wheat pathogen in North-Western Europe, and a major cause of wheat yield losses in humid climates such as UK and Ireland. This fungus is a hemibiotroph with a latency period of approximately 10-14 days. As a majority of fungicide applications to wheat are targeting *Z. tritici*, pre-symptomatic

detection would be a major advantage in disease prevention. A number of qPCR assays have been developed for *Z. tritici* (Fraaije et al., 2001, Bearchell et al., 2005, Guo et al., 2006, Abd-Elsalam et al., 2011) and although disease symptoms and pathogen load have been shown to correlate in some studies (Fraaije et al., 2001, Guo et al., 2006), there is still little known about this association, particularly in contrasting wheat genotypes.

The overall aim of this study is to investigate the relationship between visible disease symptoms and in-leaf *Z. tritici* biomass, and the consistency of this relationship over a range of wheat varieties in glasshouse experiments. Several methods of pathogen DNA standardisations were used in order to determine the accuracy of different methods.

The specific hypotheses tested in this chapter are:

1. There will be differences in visual disease symptoms between wheat genotypes
2. There will be differences in the quantification of pathogen load depending on which standardisation method is used.
3. There is a relationship between visible disease symptoms and in-leaf pathogen load for a given genotype
4. There will be differences in the visual symptoms/pathogen load relationship between genotypes.

6.2 Materials and methods

A Real-Time quantitative PCR (qPCR) assay was performed targeting the *Zymoseptoria tritici* β-tubulin gene (Bearchell et al., 2005). The qPCR quantified *Z. tritici* DNA was standardised using three different methods; one wheat nuclear target (quantified by qPCR) and two methods of quantifying total DNA; spectrophotometry (using NanoDrop 2000) and agarose gel electrophoresis. Standard curves of the *Z. tritici* β-tubulin gene assay and the wheat PKABA1 gene assay was produced using pure *Z. tritici*/wheat DNA samples, first separately and then in combinations to ensure that the assay efficiencies were not altered. The standard DNA samples had previously been quantified by a Picogreen assay (Invitrogen, USA).

Glasshouse experiments were conducted to determine the reliability of quantifying *Z. tritici* using the different methods, and to determine the relationships between quantified DNA and visual disease symptoms. In the first

experiment, two winter wheat genotypes were inoculated with a range of concentrations of *Z. tritici* spore solutions (10^4, 10^5, 10^6 spores ml^{-1}) and assessed over time. These inoculum concentrations are referred to as low, medium and high, throughout the chapter.

In the second experiment, referred to as "variety experiment", 10 genotypes were infected with equal inoculum concentration and differences in disease severities and pathogen loads were quantified.

The 16 STB isolates (originated from glycerol stocks) were cultivated on solid media prior to inoculations. Sporulating fungi was collected and diluted in distilled water to desired spore concentration. The spore solutions were sprayed on wheat seedlings until run-off and the seedlings were covered in plastic bags for 72h. In all glasshouse experiments, each pot contained five seedlings. The second leaf of each seedling was assessed separately for visual disease symptoms, and all the second leaves of the five seedlings were combined into a single sample for the molecular analysis. The analyses were performed on DNA extracted from the full leaf samples, using a CTAB DNA extraction protocol loosely based on Zhang and Stewart (2000) in combination with the standard CTAB extraction protocol of Teagasc Oak Park. The CTAB DNA extraction protocol is detailed in Chapter 3 (Materials and Methods).

All statistical analyses were performed on GenStat 14th ed (VSN International, 2011). Differences between treatments and genotypes were primarily analysed by two-way ANOVAs (with interactions). An analysis of covariance was used in order to analyse the relationships, by first determining the overall relationship by fitting a single line to the full data set. Second, the regression intercept is allowed to vary for each covariate and finally both regression intercepts and slopes are allowed to vary for each covariate. Full details of genotypes, inoculum concentrations and experimental design are presented in Chapter 3 (Materials and Methods).

6.3 Results

6.3.1. *In silico* analysis of probes and primers

In order to determine the accuracy of the probe and primer targets, a bioinformatics analysis was performed on all sequences. The probe targeting

the *Z. tritici* β-tubulin gene (Bearchell et al., 2005) had 27 matches in NCBI primer BLAST (Table 6.1). However, there were only two genes that appeared for a number of haplotypes: β-tubulin and β-tubulin like. There were two genes containing binding sites for the wheat nuclear probe (Ronning et al., 2006); W55c and a protein kinase (PKABA1; table 6.1). An alignment of W55c and PKABA1 sequences indicates 99% identity (3bp different). However, the sequences where the primer and probe binding sites are located are identical (Fig 6.1). The PKABA gene had an identical match in the EMSEMBL wheat genome assembly, while the second gene (W55c) does not have any identical matches in this database



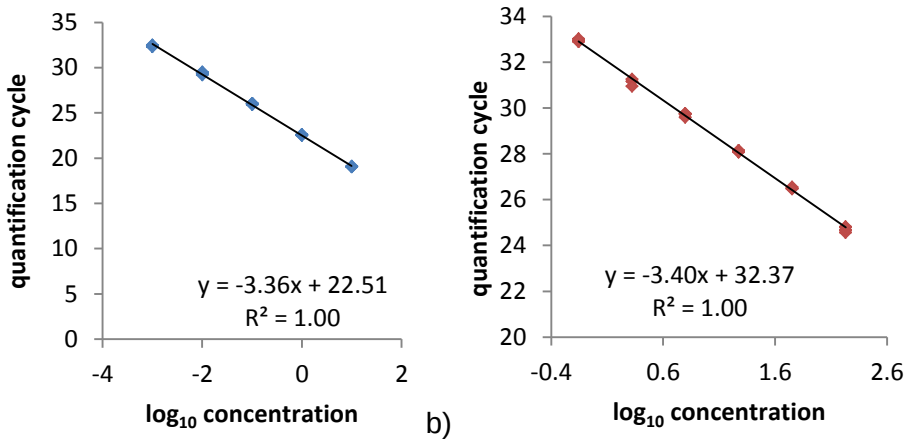
Fig 6.1. Alignment of the identical binding sites for the PKABA1 primers and probe in two genes: W55c (DQ343302.1) and the protein kinase PKABA1(M94726.1). The primers are indicated in yellow and the probe in turquoise.

Table 6.1. Targets of the probes used in this study. Shown are the results of NCBI primer- BLAST searches.

Target sequence	Hits in NCBI	No. of genes	Gene	Localisation	Accession no.
Z. tritici β-tubulin	27	2	β tubulin	Nuclear genome	AY547264.1
			β tubulin-like	Nuclear genome	AY456525.1
T. aestivum PKABA1	2	2	Serine-Threonine Protein Kinase W55c	Nuclear genome	DQ343302.1
			Protein kinase (PKABA1)	Nuclear genome	M94726.1

6.3.2. Standard curves, Limit of detection and quantification, Efficiencies

The *Z. tritici* β-tubulin gene standard curve had an r^2 value of 1.00 (Fig 6.2a), and a slope of -3.36, which equates to an efficiency of 98%. The standard curve had 5 points with a starting concentration of 11 ng diluted in 10-fold dilutions to 1.1 pg, which corresponds to 250,000 genomes diluted to 25 genomes. Hence, the limit of quantification for this assay is 1 pg (or 25 genome copies). The nuclear PKABA1 wheat gene assay had an efficiency of 97%, with a slope of -3.40 (Fig 6.2b). The sample in the standard curve was diluted 3-fold, from 170 ng to 0.7 ng (or 9,942 to 41 single genome copies). Because of this, the limit of quantification is defined at 0.7 ng or 41 haploid genomes.



a) b)

Fig. 6.2. Standard curves for a) the *Z. tritici* β-tubulin qPCR assay and b) the wheat PKABA1 gene assay.

6.3.3. Differences in symptoms and pathogen development between initial *Z. tritici* inoculum levels

Glasshouse experiments were performed to determine which method of standardising *Z. tritici* DNA is most suitable for the in-field studies. In order to do this, the relationships between pathogen load (PL) and visual disease severity were quantified over time and under different disease pressures (high, medium and low inoculum levels).

6.3.3.1. Visual disease symptoms

Disease symptoms were scored every seven days post inoculation (dpi). There were no disease symptoms in the control treatments at any of the assessments. The first symptoms appeared at 14 dpi in the medium and high inoculation levels, and at 21 dpi in the low inoculum concentration. In the first experiment, disease was scored until 28 dpi. At this time, Rialto had 76.4% symptoms in the highest concentration treatment and Xi19 had 64.4% for the same treatment (Table 6.2; Fig 6.3a, b). Although the symptoms appeared at similar times, the disease severities were considerably higher in the second experiment and assessments were finished at 21 dpi. At this time Rialto and Xi19 displayed disease symptoms of 96.5% and 99.3% in the high inoculum treatment (Table 6.3; Fig. 6.4a, b). The disease symptoms in the low and medium inoculum concentrations were also considerably higher in the second inoculum concentration experiment. The disease symptoms of Rialto at the final assessment increased from 1.7% in the first experiment to 10.3% in the second for the low concentrations, and Xi19 from 2.7% to 23.1% in the second experiment. In the medium concentration, the disease symptoms increased from 18.6% for both Rialto and Xi19 to 54.0% and 79.5% respectively, at 21 dpi (Table 6.2-3). Due to the large differences between the disease severities ($p<0.001$) the experiments were analysed separately.

The overall disease symptoms increased with time; when disease symptoms were plotted against time, days post-inoculation accounted for 26.1% and 64.2% ($p<0.001$; first and second experiment respectively) of variation in disease symptoms. There were significant differences between inoculum levels when the regression intercepts and slopes were allowed to vary; this model accounted for 90.5% and 96.5% of the variation of disease symptoms for the first and second experiment, respectively ($p<0.001$).

In both experiments, the high and medium concentrations caused increased symptom expression and symptom development rate compared to the low concentrations ($p<0.001$). In the first inoculum concentration experiment there was a strong increase in the symptom expression and development rate of the high inoculum concentration compared to the medium concentration ($p<0.001$; fig 6.3a-b), and there was a similar response in the second experiment although slightly weaker ($p=0.029$; fig 6.4a-b).

Table 6.2. Visual disease symptom scores of the second leaves as percentages in the first inoculation experiment. The scores are reported by genotype, assessment date, and for the low, medium and high concentrations (10^4, 10^5, 10^6 spores ml^{-1}). Two-way ANOVAs were carried out on log-transformed values, reported in brackets.

Concentration	**0 dpi**	**7 dpi**	**14 dpi**	**21 dpi**	**28 dpi**
Rialto					
Control	0	0	0.0	0.0	0.0
Low	0	0	0.0	0.3 (0.10)	1.7 (0.39)
Medium	0	0	1.4 (0.30)	9.5 (0.94)	18.6 (1.28)
High	0	0	13.4 (1.16)	40.8 (1.60)	76.4 (1.89)
Xi19					
Control	0	0	0.0	0.0	0.0
Low	0	0	0.0	1.0 (0.26)	2.7 (0.51)
Medium	0	0	2.3 (0.55)	11.3 (1.07)	18.6 (1.28)
High	0	0	12.1(1.11)	56.8 (1.74)	64.4 (1.82)
P concentration	**NS**	**NS**	**<0.001**	**<0.001**	**<0.001**
SED (df)			0.094 (18)	0.0769 (18)	0.0769 (18)
P genotype	**NS**	**NS**	**NS**	**0.032**	**NS**
SED (df)				0.0628 (18)	
P conc.genotype	**NS**	**NS**	**NS**	**NS**	**NS**
SED (df)					

Table 6.3. Visual disease symptom scores of the second leaves as percentages in the second inoculation experiment. The scores are reported by genotype, assessment date, and for the low, medium and high concentrations (10^4, 10^5, 10^6 spores ml^{-1}). Two-way ANOVAs were carried out on log-transformed values, reported in brackets.

Concentration	0 dpi	7 dpi	14 dpi	21 dpi
Rialto				
Control	0	0	0.0	0.0
Low	0	0	0.0	10.3 (1.07)
Medium	0	0	0.6 (0.19)	54.0 (1.74)
High	0	0	6.4 (0.85)	96.5 (1.99)
Xi19				
Control	0	0	0.0	0.0
Low	0	0	0.0	23.1 (1.36)
Medium	0	0	1.4 (0.47)	79.5 (1.90)
High	0	0	9.2 (0.95)	99.3 (2.00)
P concentration	**NS**	**NS**	**<0.001**	**0.001**
SED (df)			0.115 (18)	0.0395 (18)
P genotype	**NS**	**NS**	**NS**	**<0.001**
SED (df)				0.0323 (18)
P conc.genotype	**NS**	**NS**	**NS**	**0.011**
SED (df)				0.0560 (18)

6.3.3.2. Quantifying in-leaf pathogen load

Pathogen load (PL), the in-leaf pathogen DNA, was quantified by the *Z. tritici* β-tubulin gene and standardised by three different methods. The first method, in which both *Z. tritici* DNA and wheat DNA were quantified in a multiplex qPCR reaction is referred to as PL_{MP} throughout the chapter. In the other two methods, the qPCR quantified *Z. tritici* DNA was standardised against total DNA quantified by NanoDrop (PL_{ND}) and gel electrophoresis (PL_{GE}).

In the first experiment, the average pathogen load in the multiplex qPCR-standardised samples (PL_{MP}) was 8.4 pg *Z.tritici* ng wheat DNA^{-1}. When pathogen load was standardised by total DNA, the NanoDrop method (PL_{ND}) yielded an average 5.7 pg *Z. tritici* ng total DNA^{-1} and the gel electrophoresis method (PL_{GE}) 11.7 pg *Z. tritici* ng total DNA^{-1}. In the second experiment, as for the visual disease symptoms, the average pathogen loads were considerably higher for all methods. The average PL_{MP} was 24.3 pg *Z. tritici* ng wheat DNA^{-1}, while PL_{GE} and PL_{ND} averaged 22.6 and 13.9 pg *Zt* ng total DNA^{-1} respectively.

At 7 dpi, there was a difference between the high inoculum concentration and the two lower inoculum levels for PL_{ND} in the first experiment ($p<0.001$). PL_{MP} also displayed near-significant trends for this ($p=0.058$), but PL_{GE} did not. There were no differences between genotypes, or interactions between genotypes and inoculum levels. In the second experiment at 7 dpi the differences between inoculum levels were significant in all methods ($p<0.001$). At 14 dpi in both experiments all methods differentiated between inoculum levels. The highest significance levels were displayed for PL_{MP} ($p<0.001$), but the PL_{GE} and PL_{ND} also showed differences between inoculum levels in both experiments (Table 6.4). At the following sampling dates, 21 and 28 dpi, all methods displayed highly significant differences between inoculum levels in the first experiment ($p<0.001$; Table 6.4).

PL_{MP} displayed a near-significant difference between the two genotypes at 14 dpi in the first experiment ($p=0.077$) and a difference in the second ($p<0.001$), but there was no inoculum level x genotype interaction. This method did not show differences between genotypes at 21 dpi in either experiment, but had a significant inoculum level x genotype interaction in the second experiment ($p=0.005$). At 28 dpi, there were significant differences between genotypes ($p=0.002$) and an inoculum level x genotype effect ($p=0.028$).

PL_{ND} displayed significant differences between genotypes at 14, 21 and 28 dpi in the first experiment (table 6.4), but did not indicate any differences in the second experiment. There was no inoculum level x genotype interaction at any of the time points.

PL_{GE} displayed differences between genotypes at 28 dpi in the first experiment ($p=0.009$) and at 7 dpi and 14 dpi in the second ($p=0.007$). This method also displayed significant inoculum level x genotype interactions at all sampling dates in the second experiment (Table 6.4), but only a near-significant interaction at 28 dpi in the first experiment ($p=0.063$). It should be noted that only 52 out of 72 samples were successfully quantified using this method in the second experiment, as the remaining samples could not be visualised on the agarose gel.

Table 6.4. Pathogen loads quantified by the standardisation methods. The average pathogen loads (ln pg *Z tritici* per ng DNA) are reported for the two experiments. The two genotypes, assessment dates and inoculum levels (10^4, 10^5, 10^6 spores ml^{-1}) are reported separately. The significant ($p<0.05$) and near significant ($p<0.1$) values are reported as analysed by a two-way ANOVA.

		Experiment 1				Experiment 2		
Multiplex qPCR	**Inoculum**	**7 dpi**	**14 dpi**	**21 dpi**	**28 dpi**	**7 dpi**	**14 dpi**	**21 dpi**
Rialto	Low	0.14	0.17	0.27	0.51	0.05	0.51	1.89
	Medium	0.20	0.50	1.39	1.99	0.08	1.00	3.26
	High	0.39	1.56	3.23	4.39	0.27	1.64	5.11
Xi19	Low	0.18	0.17	0.54	0.35	0.05	0.87	2.24
	Medium	0.06	1.23	1.49	1.45	0.13	1.63	4.16
	High	0.26	1.60	3.33	2.80	0.27	2.28	4.19
	P inoculum	0.058	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	sed, df	0.081 (15)	0.243 (13)	0.204 (15)	0.246 (15)	0.041 (13)	0.219 (9)	0.243 (14)
	P genotype	NS	0.077	NS	0.002	NS	0.003	NS
	sed, df		0.202 (13)		0.201 (15)		0.176 (9)	
	P G x I	NS	NS	NS	0.028	NS	NS	0.005
	sed, df				0.347 (15)			0.343 (14)
		Experiment 1				**Experiment 2**		
NanoDrop	**Inoculum**	**7 dpi**	**14 dpi**	**21 dpi**	**28 dpi**	**7 dpi**	**14 dpi**	**21 dpi**
Rialto	Low	0.01	0.01	0.03	0.18	0.01	0.03	0.74
	Medium	0.01	0.07	0.34	2.27	0.01	0.26	3.60
	High	0.03	0.59	2.53	3.78	0.05	0.70	4.12
Xi19	Low	0.01	0.02	0.17	0.22	0.01	0.18	1.99
	Medium	0.01	0.43	1.12	1.59	0.02	0.49	3.92
	High	0.04	1.41	3.11	3.24	0.06	1.40	3.42
	P inoculum	<0.001	0.005	<0.001	<0.001	<0.001	0.022	<0.001
	sed (df)	0.005 (15)	0.263 (13)	0.266 (15)	0.213 (15)	0.0062 (13)	0.347 (9)	0.322 (14)
	P genotype	NS	0.033	0.036	0.040	NS	NS	NS
	sed (df)		0.219 (13)	0.217 (15)	0.174 (15)			
	P G x I	NS	NS	NS	NS	NS	NS	0.033
	sed (df)							0.456 (14)

Table 6.4 continued

		Experiment 1				Experiment 2		
Gel electrophoresis	**Inoculum**	**7 dpi**	**14 dpi**	**21 dpi**	**28 dpi**	**7 dpi**	**14 dpi**	**21 dpi**
Rialto	Low	0.03	0.03	0.15	0.51	0.06	0.16	1.82
	Medium	0.04	0.20	0.69	2.62	0.07	0.91	3.84
	High	0.07	1.10	2.19	5.05	0.33	1.56	5.10
Xi19	Low	0.05	0.03	0.32	0.42	0.08	0.30	2.15
	Medium	0.02	0.71	1.00	2.05	0.10	0.99	4.67
	High	0.08	1.01	3.02	3.35	0.32	0.69	3.94
	P inoculum	0.086	0.003	<0.001	<0.001	0.002	<0.001	<0.001
	sed (df)	0.019 (15)	0.240 (13)	0.332 (15)	0.321 (15)	0.061 (12)	0.074 (4)	0.290 (6)
	P genotype	NS	NS	NS	0.009	NS	0.007	NS
	sed (df)				0.262 (15)		0.044(4)	
	P G x I	NS	NS	NS	0.063	NS	0.003	0.024
	sed (df)				0.454 (15)		0.104 (4)	0.431(6)

6.3.3.3 Quantifying pathogen load progression

There were highly significant differences between the treatments (inoculum levels), days post inoculation (dpi) and a strong inoculum x dpi interaction for all methods in both experiments ($p<0.001$). This was also true when Rialto was analysed separately; the differences between inoculum level, dpi and the interaction between these were still highly significant for all three methods ($p<0.001$). All these differences were also highly significant ($p<0.001$) for Xi19 in the first experiment for all methods, and in the second experiment only for PL_{MP}. In the second experiment, Xi19 PL_{GE} and PL_{ND} displayed differences between inoculum levels ($p<0.001$) and dpi ($p<0.001$), and inoculum x dpi interactions ($p=0.015$ and $p=0.014$, respectively).

In the first experiment, the overall relationship between PL_{MP} and dpi accounted for 28.3% of the variation ($p<0.001$). When the regression intercepts and slopes for each inoculum level were allowed to vary independently, the model showed that dpi accounted for 83.7% of variation in PL_{MP}, hence displaying a highly significant inoculum level interaction within this relationship. The high and medium inoculum levels displayed increased PL progression rates compared to the lowest inoculum concentration ($p<0.001$). In addition, the high inoculum level displayed significantly higher PL progression rate compared to the medium inoculation concentration ($p<0.001$).

The same trend was found when each genotype was analysed separately. In the first experiment, there was an overall relationship between PL_{MP} and dpi accounting for 33.1% for Rialto ($p<0.001$). When inoculum level was introduced as a covariate and the regression intercepts and slopes were allowed to vary, this model accounted for 95.1% of variation in PL_{MP} ($p<0.001$; fig 6.3a), displaying a strong covariate interaction in the pathogen load – dpi relationship. There was also an overall relationship between pathogen load and dpi for Xi19, accounting for 22.1% of variation in PL_{MP} ($p<0.001$). When the inoculum level regression intercepts and slopes were allowed to vary the model accounted for 70.8% of the variation and displayed a highly significant covariate interaction ($p<0.001$; fig 6.3c). The highest inoculum concentration displayed increased PL progression rates compared to the low and medium concentration for both genotypes, but more so for Rialto ($p<0.001$) than Xi19 ($p=0.043$). For Rialto, the medium concentration level had increased PL

progression rate compared to the lowest concentration ($p<0.001$), but not for Xi19 ($p=0.063$).

The overall relationship between PL_{MP} and dpi was much stronger in the second experiment at 74.7% ($p<0.001$), but when the concentration level regression slopes and intercepts varied the relationship increased further to 92.0% ($p<0.001$). Hence, there was a highly significant inoculum concentration interaction in this relationship ($p<0.001$). The high and medium inoculum concentrations displayed significantly increased PL progression rates compared to the low concentration ($p<0.001$), but there was only a near-significant difference between the high and medium concentration progression rates ($p=0.058$).

There was a strong overall relationship between PL_{MP} and dpi for Rialto, accounting for 70.5% of the variation in $PL_{MP.}$ When inoculum level intercepts and slopes were allowed to vary, the model accounted for 92.9% and therefore displayed a strong inoculum level interaction in this relationship ($p<0.001$; Fig 6.3b). Both the high and the medium inoculum levels had significantly higher PL progression rate compared to the lowest concentration ($p<0.001$ and $p=0.007$, respectively), and in turn, the highest inoculum level displayed an increased PL progression rate compared to the medium inoculum level ($p=0.005$). There was also a strong overall relationship between PL_{MP} and dpi for Xi19 accounting for 79.0% of the variation in $PL_{MP.}$ When inoculum level intercepts and slopes were allowed to vary, the model accounted for 94.2% and displayed a strong inoculum level interaction in this relationship ($p<0.001$; Fig 6.3d). Both the high and the medium inoculum levels had significantly higher PL progression rate compared to the lowest concentration ($p<0.001$), but there was no difference between these two concentrations.

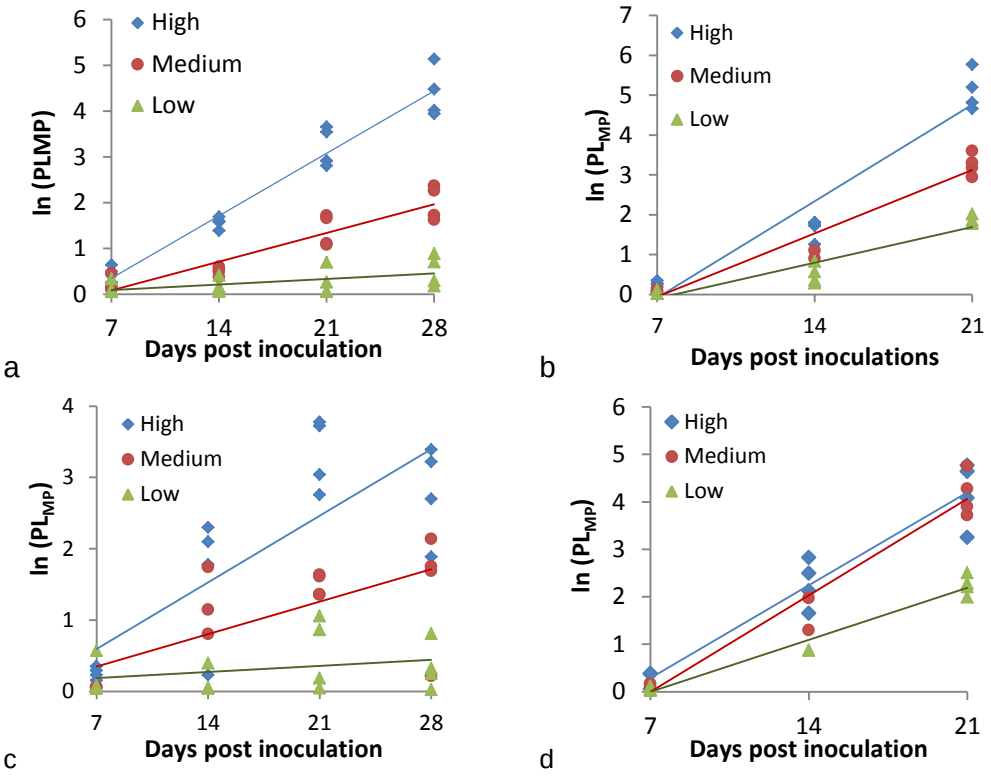


Fig 6.3. The linear regressions between pathogen loads and days post inoculation for three inoculum concentrations using in the multiplex qPCR method (PL_{MP}). The days post inoculation is shown on the x-axis and the pathogen loads (the natural logarithm of pg *Z. tritici* ng wheat^{-1}) are shown on the y-axis. a) Rialto in experiment 1; b) Rialto in experiment 2; c) Xi19 in experiment 1; d) Xi19 in experiment 2.

The overall relationship between days post-inoculation and PL_{ND} in the first experiment accounted for 32.2% of the variation ($p<0.001$), which increased to account for 84.8% when inoculum level slopes and intercepts were allowed to vary ($p<0.001$). The high and medium inoculum levels displayed increased PL progression rates compared to the lowest concentration ($p<0.001$), and the PL progression rate increased in the highest inoculum level compared to the medium inoculum level ($p<0.001$).

Rialto displayed an overall relationship between PL_{ND} and dpi, accounting for 36.5% of variation ($p<0.001$). When inoculum level regression intercepts and slopes varied, the model accounted for 87.9% of the variation and displayed a strong inoculum level interaction in the relationship ($p<0.001$; Fig 6.4a). There were significant differences between all inoculum levels ($p<0.001$), with PL progression rate increasing as inoculum levels increased. Xi19 had an overall relationship between PL_{ND} and dpi, accounting for 26.6% of variation ($p<0.001$). When inoculum level regression intercepts and slopes

varied, the model accounted for 81.1% of the variation and therefore displayed a strong inoculum level interaction in the relationship ($p<0.001$; Fig 6.4c). Both the high and medium inoculum concentrations displayed increased PL progression rates compared to the low concentration ($p<0.001$ and $p=0.010$, respectively), and the high inoculum concentration had an increased PL progression rate compared to the medium concentration ($p=0.009$).

The second experiment displayed an overall relationship between PL_{ND} and dpi accounting for 64.5% ($p<0.001$). This increased to 81.4% when the analysis allowed the regression intercepts and slopes for each inoculum concentration to vary ($p<0.001$). There were significant increases in PL progression rate in the high and medium inoculum levels compared to the low concentration ($p<0.001$), but not between these two inoculum levels.

There was an overall relationship between PL_{ND} and dpi for Rialto accounting for 57.7% ($p<0.001$), which increased to 83.4% when the inoculum level regression slopes and intercepts were allowed to vary ($p<0.001$; Fig 6.4b). The high and medium inoculum levels displayed increased PL progression rate compared to the lowest concentration ($p<0.001$), but there was no difference between these two levels. Xi19 displayed an overall relationship accounting for 71.6% of the variation ($p<0.001$). When inoculum level regression intercepts and slopes were allowed to vary, the model accounted for 80.6% of variation in PL_{ND} and hence displayed an inoculum level interaction in the relationship ($p=0.029$; Fig 6.4d). The medium concentration had increased pathogen load progression rate compared to the low concentration ($p=0.011$) and also an increased rate compared to the high concentration, but this was not significant. There was only a near-significant increase in PL progression rate in the high concentration compared to the low ($p=0.062$).

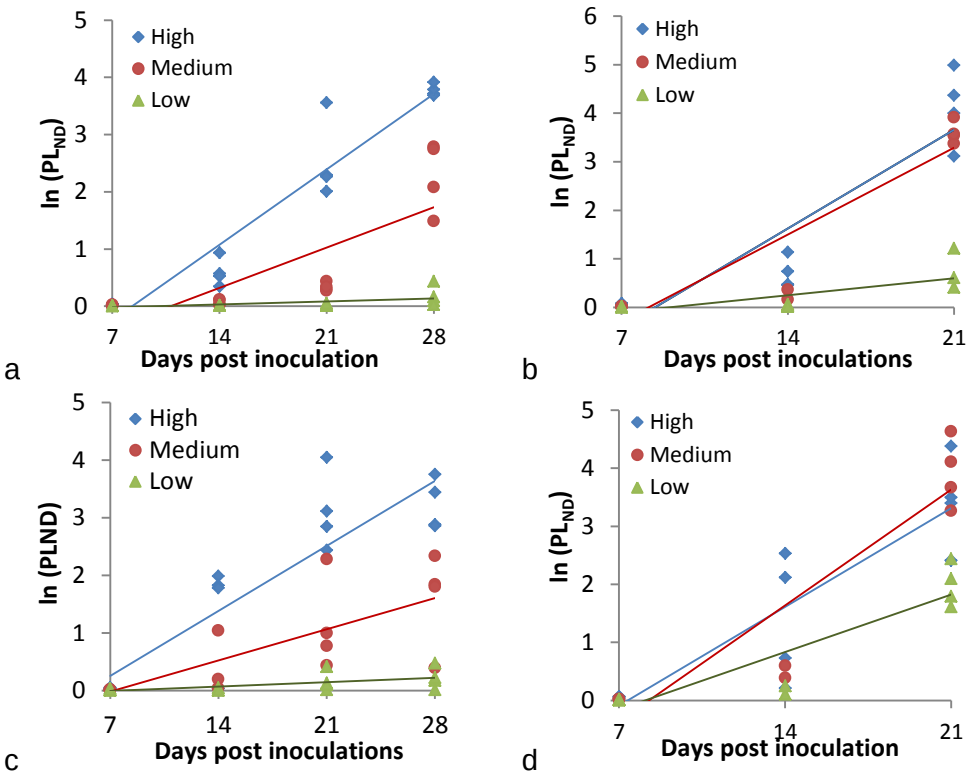


Fig 6.4. The linear regressions between pathogen loads and days post inoculation for three inoculum concentrations using in the NanoDrop method (PL_{ND}). The days post inoculation is shown on the x-axis and the pathogen loads (the natural logarithm of pg Z. tritici ng total DNA^{-1}) are shown on the y-axis. a) Rialto in experiment 1; b) Rialto in experiment 2; c) Xi19 in experiment 1; d) Xi19 in experiment 2.

The third method, PL_{GE}, displayed an overall relationship with days post inoculation in the first experiment, accounting for 36.5% ($p<0.001$). This, increased to 81.5% when the inoculum level regression slopes and intercepts were allowed to vary ($p<0.001$). There were significant differences in PL progression rate between all inoculum levels ($p<0.001$) with an increase in progression rate related to increased inoculum level. There was an overall relationship between pathogen load and dpi for Rialto accounting for 36.5% ($p<0.001$), which increased to 85.0% when inoculum level regression intercepts and slopes were allowed to vary ($p<0.001$; Fig 6.5a). There were again significant differences between all inoculum levels, particularly between the high and medium inoculum levels and the low inoculum level ($p<0.001$) but also between the high and medium inoculum levels ($p=0.002$). There was an overall relationship between PL_{GE} and dpi for Xi19 accounting for 33.1% of the PL variation ($p<0.001$). This increased to 77.2% when inoculum level regression intercepts and slopes were allowed to vary ($p<0.001$; Fig 6.5c). There were significant differences between the

inoculum level PL progression rates, particularly between the high and low inoculum concentration ($p<0.001$) but also between the high and medium ($p=0.024$) and low and medium inoculum levels ($p=0.013$).

The relationships were stronger in the second experiment. The pathogen load – dpi relationship accounted for 75.1% ($p<0.001$) and increased to 87.0% when the inoculum level regression slopes and intercepts varied individually. When analysed separately these regression models accounted for 91.4% and 83.2% of the variation in pathogen load for Rialto and Xi19 respectively ($p<0.001$; Fig 6.5b,d). It should be noted that a large portion of the data set is missing for this method as a number of samples did not produce visible bands on the gels, possibly due to low concentration and/or low sample quality. Most of these samples were, however, successfully quantified using the other methods.

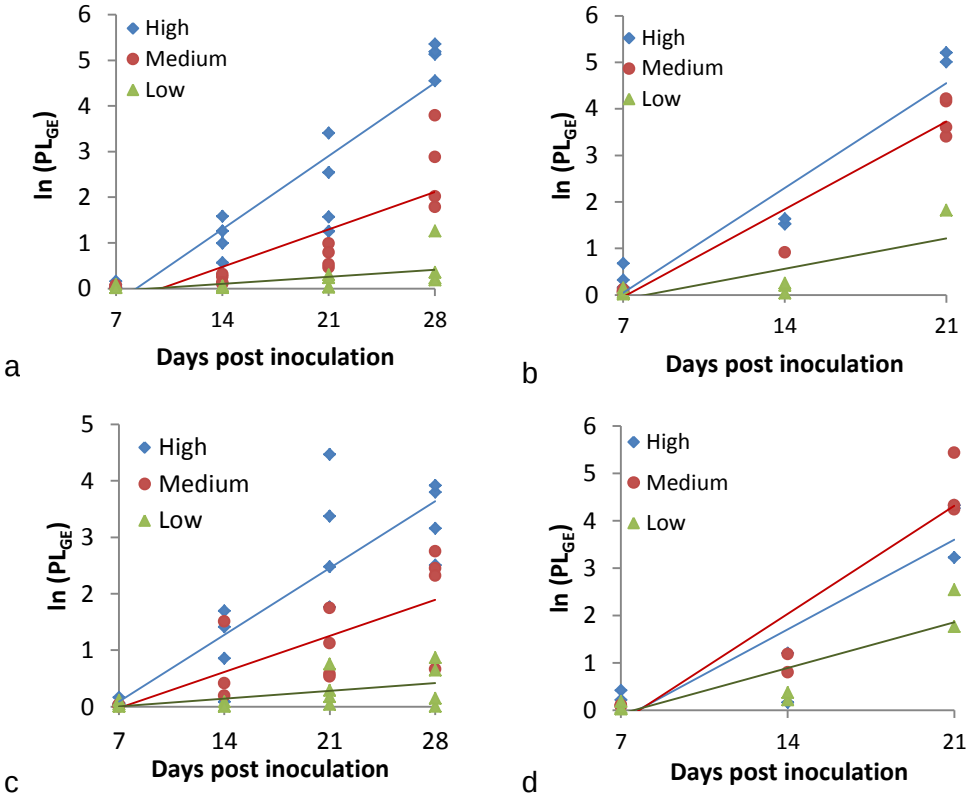


Fig 6.5. The linear regressions between pathogen loads and days post inoculation for three inoculum concentrations in the gel electrophoresis method (PL_{GE}). The days post inoculation is shown on the x-axis and the pathogen loads (the natural logarithm of pg Z. tritici ng total DNA^{-1}) are shown on the y-axis. a) Rialto in experiment 1; b) Rialto in experiment 2; c) Xi19 in experiment 1; d) Xi19 in experiment 2.

6.3.3.4 The relationship between pathogen load and disease severity

The pathogen loads standardised by multiplex PCR (PL_{MP}) explained 78.6% of the variation in disease symptoms in the first experiment ($p<0.001$; Fig 6.6a). There was no improvement in further adding to the regression model by varying genotype intercepts, or both intercepts and slopes. In the second experiment, the relationship between PL_{MP} and disease severity was slightly stronger with an r^2 value of 83.5% ($p<0.001$; Fig 6.6b). There was no significant increase when the genotype regression slopes and intercepts were allowed to vary.

The relationship between pathogen load and disease severity in the samples standardised by NanoDrop (PL_{ND}) accounted for 83.2% of the variation in the first glasshouse experiment ($p<0.001$; Fig 6.6c). The relationship was similar in the second experiment, pathogen load accounting for 84.2% of the disease symptoms ($p<0.001$; Fig 6.6d). There was no significant improvement when including genotypes as groups in the regression analysis.

The pathogen loads standardised by gel electrophoresis (PL_{GE}) displayed the weakest relationship with disease symptoms in the first experiment, accounting for 80.2% of the variation ($p<0.001$; Fig 6.6e). In the second experiment the overall relationship accounted for 59.6% ($p<0.001$; Fig 6.6f), and there was no additional benefit of adding genotypes as groups in either analysis.

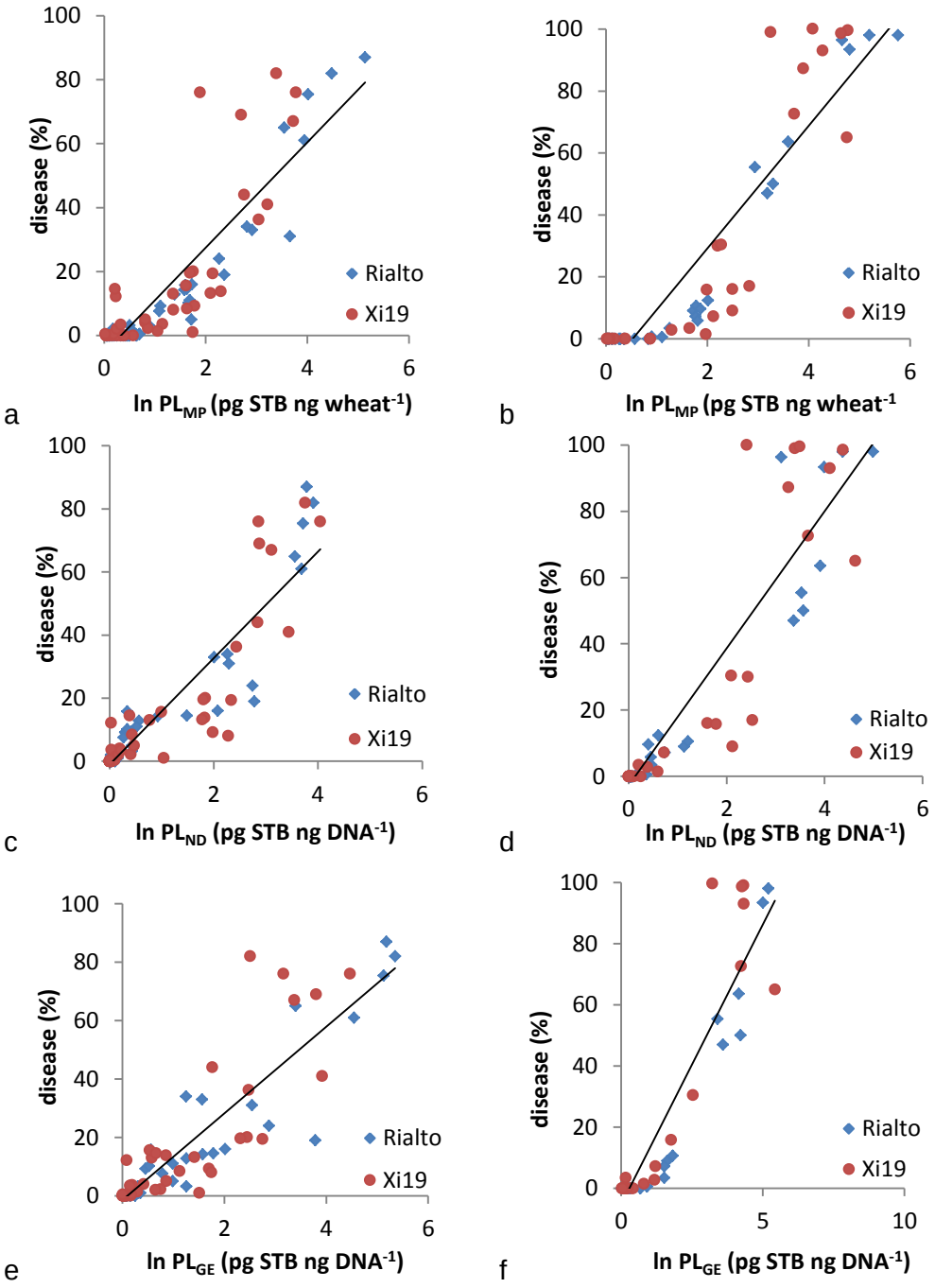


Fig 6.6. The relationship between pathogen load and disease severity. a) The relationship between PL_{MP} and disease severity in the first experiment ($p<0.001$, $r^2=0.786$). b) The relationship between PL_{MP} and disease severity in the second experiment ($p<0.001$; $r^2=0.835$). c) The relationship between PL_{ND} and disease severity in the first experiment ($p<0.001$ $r^2=0.832$). d) The relationship between PL_{ND} and disease severity in the second experiment ($p<0.001$ $r^2=0.842$). e) The relationship between PL_{GE} and disease severity in the first experiment ($p<0.001$, $r^2=0.802$. f) The relationship between PL_{GE} and disease severity in the second experiment ($p<0.007$, $r^2=0.596$). All pathogen loads are transformed using natural logarithms.

6.3.4. Determining the relationship between pathogen load and visual symptoms over a range of genotypes.

In order to determine the relationships between pathogen load and visual disease symptoms in an extended range of wheat genetic backgrounds, an additional experiment was carried out using 10 different genotypes selected for contrasting genetic backgrounds and resistance to Septoria tritici blotch (Table 3.5 in Chapter 3 – Materials and Methods).

6.3.4.1 Visual disease symptoms

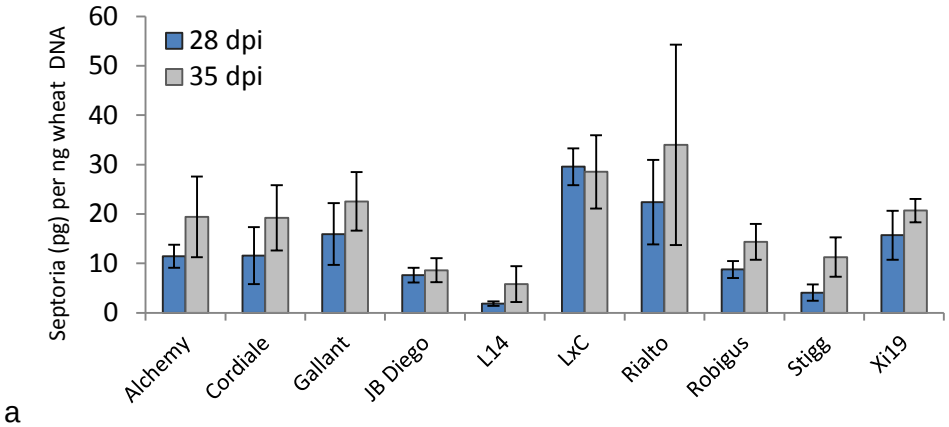
The visual disease symptoms were assessed at 28 dpi and 35 dpi, and the symptoms increased significantly between these; from an average score of 72.5% to 89.4% ($p<0.001$). There were also significant differences in disease severity between genotypes at each timing ($p<0.001$), with disease symptom ranges of 38.8% - 87.1% and 56.5% – 98.1% at 28 and 35 dpi, respectively (Table 6.5). The two genotypes displaying the lowest symptom areas at both assessments were the CIMMYT-derived L14xR127 and the UK variety Stigg (Table 6.5). At 28 dpi, L14xR127 was significantly less diseased than 4 out of 10 genotypes while Stigg had significantly less disease than the most diseased genotype (Alchemy). At 35 dpi, L14x127 and Stigg had significantly less disease than all other genotypes (Table 6.5). The disease symptoms at the two assessments were compared to the resistance ratings of the commercial varieties, and with the exception of Stigg (resistance rating 7) there was little agreement between symptoms and ratings (Appendix Fig 6.1a, b).

6.3.5.2 Disease detection in 10 wheat genotypes

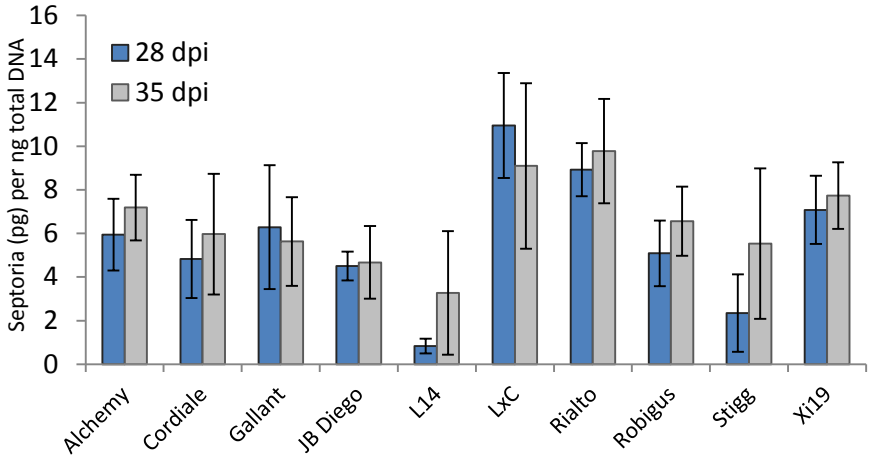
The two most successful methods used in the previous glasshouse experiments were selected for the pathogen load quantification; the multiplex qPCR (PL_{MP}) and total DNA by NanoDrop (PL_{ND}). A one-way ANOVA determined that there were differences in pathogen load between sampling dates when standardised with multiplex qPCR ($p=0.014$) but not when standardised with NanoDrop quantified DNA ($p=0.20$). Both methods were, however, able to differentiate pathogen loads between varieties at both sampling dates ($p<0.001$; Table 6.5; Fig 6.7a, b).

At 28 dpi, PL_{MP} ranged between 1.9 to 29.6 pg *Z tritici* ng wheat DNA^{-1} and between 0.8 – 11.0 pg *Z. tritici* ng total DNA^{-1} for PL_{ND} ($p<0.001$; Table 6.5). At 35 dpi, the ranges were slightly smaller; 5.8 – 28.5 pg *Z tritici* ng

wheat^{-1} ($p<0.001$) and 3.3 – 9.1 pg *Z. tritici* ng total DNA^{-1} ($p=0.008$), for PL_{MP} and PL_{ND} respectively.



a



b

Fig 6.7. Pathogen loads of the 10 genotypes at 28 and 35 dpi as standardised by a) multiplex qPCR and b) total DNA quantified with NanoDrop.

Table 6.5. Average disease severities and pathogen loads at 28 and 35 days post inoculation on genotypes screened in the variety glass house experiment. Genotype differences within each assessment date were analysed by one-way ANOVA, and the genotypes were grouped according to Bonferroni multiple comparison tests.

	28 dpi			35 dpi		
Variety	**Symptoms (%)**	**ln PL_{MP} pg STB ng wheat^{-1}**	**ln PL_{ND} pg STB ng DNA^{-1}**	**Symptoms (%)**	**ln PL_{MP} pg STB ng wheat^{-1}**	**ln PL_{ND} pg STB ng DNA^{-1}**
Alchemy	87.1 c	2.50 cd	1.91 cd	94.0 b	2.94 bcd	2.08 ab
Cordiale	70.9 abc	2.44 cd	1.72 bc	95.3 b	2.95 bcd	1.85 ab
Gallant	79.3 abc	2.76 cde	1.89 cd	97.7 b	3.13 cd	1.85 ab
JB Diego	84.6 bc	2.14 bc	1.70 bc	92.9 b	2.23 ab	1.69 ab
L14xR127	38.8 a	1.05 a	0.59 a	56.5 a	1.78 a	1.22 a
Cadenza x Lynx 5H	85.1 bc	3.41 e	2.46 d	94.0 b	3.34 d	2.24 b
Rialto	81.9 bc	3.04 de	2.29 cd	96.1 b	3.40 d	2.35 b
Robigus	74.5 abc	2.26 bc	1.77 bcd	97.0 b	2.70 bcd	1.99 ab
Stigg	45.2 ab	1.58 ab	1.08 ab	72.6 a	2.45 abc	1.69 ab
Xi19	77.5 abc	2.77cde	2.07 cd	98.1 cb	3.07 cd	2.15 ab
Average	**72.5**	**2.41**	**1.76**	**89.4**	**2.78**	**1.91**
P genotype	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**
SED (df)	**11.34 (36)**	**0.205 (38)**	**0.207 (38)**	**5.90 (47)**	**0.234 (47)**	**0.269 (47)**

6.3.4.3 Pathogen loads vs. disease symptoms

The relationships between pathogen load and disease symptoms were determined for the two methods. The PL_{MP} displayed an overall relationship with visual disease symptoms, accounting for 25.8% of the variation ($p<0.001$). This improved when the pathogen loads were transformed as the natural logarithm (ln, base *e*), accounting for 48.8% of the variation with a linear regression ($p<0.001$; Fig 6.8). The relationship between visual symptoms and PL_{ND} was also significant ($p<0.001$), and accounted for 30.4% of the symptoms variation, which improved to 43.4% when transformed ($p<0.001$; Fig 6.8). Due to this, the pathogen load data in all following regression analyses are transformed with the natural logarithm.

There were differences in the overall symptom expression - pathogen load relationships at the two sampling dates. The relationships were highly significant at 28 dpi ($p<0.001$), but PL_{ND} had a slightly better fit with 47.9% compared to 39.6% for PL_{MP} (Fig 6.9a), In contrast, PL_{MP} had a better fit at 35 dpi accounting for 51.4% of variation in symptoms, compared to 39.6% for PL_{ND} (Fig 6.9b).

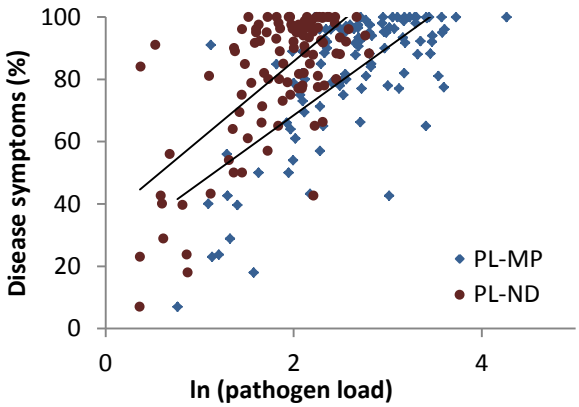


Fig 6.8. Relationships between pathogen load and visual disease symptoms. The linear relationships ($p<0.001$) between disease symptoms and ln-transformed pathogen load standardised by multiplex qPCR ($r^2=0.488$) and NanoDrop ($r^2=0.434$).

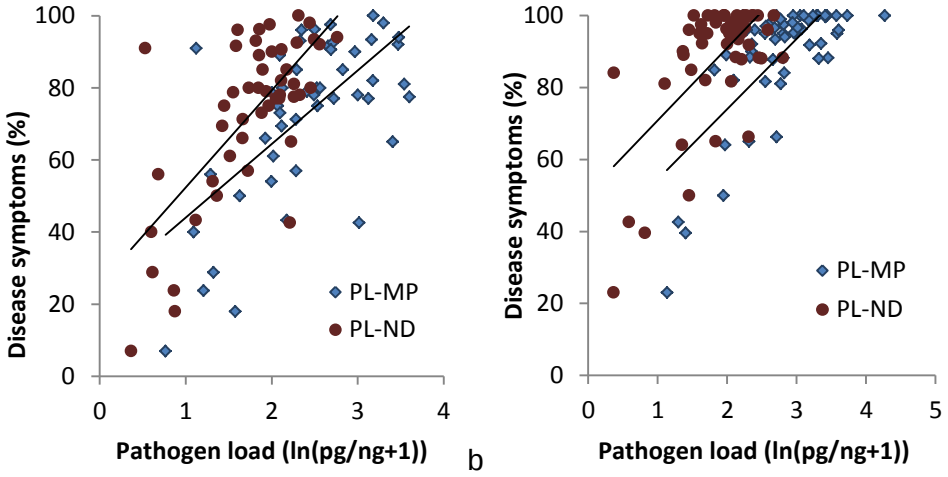


Fig 6.9. The linear relationships between pathogen load and visual disease symptoms ($p<0.001$) at a) 28 dpi for multiplex qPCR ($r^2=0.396$) and NanoDrop ($r^2=0.479$) and b) at 35 dpi for multiplex qPCR ($r^2=0.514$) and NanoDrop ($r^2=0.396$).

6.3.4.4 Genotype differences in the relationship between pathogen load and disease symptoms

There was large variation in the relationship between pathogen load and visual disease symptoms between the 10 genotypes. Although the overall relationships with the combined data sets were quite similar and highly significant ($p<0.001$), there were genotype differences in the pathogen load-visual symptoms relationships (Figs 6.10-11). As described in the previous section, the overall relationship between disease symptoms and PL_{MP} accounted for 48.8% of the symptom variation ($p<0.001$). When allowing genotype intercepts to vary, the parallel regression model accounted for 60.7% of the variation ($p<0.001$; Table 6.6). The genotype Alchemy was used as a reference variety, and the parallel regression indicated that JB Diego had a significantly higher disease symptom expression for the same level of pathogen load ($p=0.045$). On the other hand, Cadenza x Lynx 5H (CxL), Rialto and Stigg had significantly less disease symptoms for any level of pathogen load than Alchemy ($p=0.007$, $p=0.016$ and $p=0.025$, respectively).

The PL_{ND} data set increased from accounting for 43.4% of the variation to 50.8% when genotype regression intercepts were allowed to vary ($p=0.007$; table 6.7). There was no additional benefit to allow genotype regression slopes to vary. The parallel regression indicated that Stigg and L14xR127 had

significantly lower disease expressions for any level of pathogen load compared to Alchemy ($p=0.006$ and $p=0.009$, respectively).

When the two assessment dates were analysed separately, there was no improvement by adding genotypes as groups in the regression analysis at 28 dpi for either method of quantifying pathogen load. However, at 35 dpi the relationship between PL_{MP} and disease symptoms improved from 51.4% to 74.2% ($p<0.001$) when genotype regression intercepts were allowed to vary, and to 86.3% when the slopes were also allowed to vary ($p<0.001$). Two of the genotypes displayed an increased rate of disease symptom development per unit pathogen load compared to Alchemy: L14xR127 and Stigg ($p<0.001$ and $p=0.029$, respectively). However, this could be because of initially lower disease symptoms than the other genotypes.

The relationship between PL_{ND} and disease symptoms at 35 dpi increased from 39.6% to 69.7% when genotype regression intercepts were allowed to vary, and further to account for 84.0% of variation when varying genotype regression slopes ($p<0.001$). With this method, L14xR127 displayed an increased symptom development rate for each pathogen load unit compared to Alchemy ($p=0.008$), while Stigg did not.

Table 6.6. The linear regression between visual disease symptoms and pathogen load (standardised with multiplex qPCR and ln-transformed), with parallel regressions fitted for each genotype. The reference genotype is Alchemy (linear function y = 24.8x + 22.84), with p-values referring to significant differences from this slope.

Genotype	Slope (%STB ln(PL_{MP} +1))	P-value
Alchemy	24.80	<.001
Cordiale	18.72	0.281
Gallant	17.68	0.234
JB Diego	36.77	**0.045**
L14xR127	15.20	0.174
Cadenza x Lynx 5H	8.26	**0.007**
Rialto	9.74	**0.016**
Robigus	26.66	0.743
Stigg	11.10	**0.025**
Xi19	17.94	0.227

Table 6.7. The linear regression between visual disease symptoms and pathogen load (standardised with total DNA and ln-transformed), with parallel slopes fitted for each genotype. The reference genotype is Alchemy (linear function y = 20.0x + 50.72), with p-values referring to significant differences from this slope.

Genotype	Slope (% STB ln(PL_{ND} +1))	P-value
Alchemy	20.00	<.001
Cordiale	17.67	0.714
Gallant	20.01	0.999
JB Diego	24.48	0.484
L14xR127	0.03	**0.009**
Cadenza x Lynx 5H	12.44	0.240
Rialto	11.39	0.203
Robigus	18.18	0.773
Stigg	1.14	**0.006**
Xi19	15.76	0.501

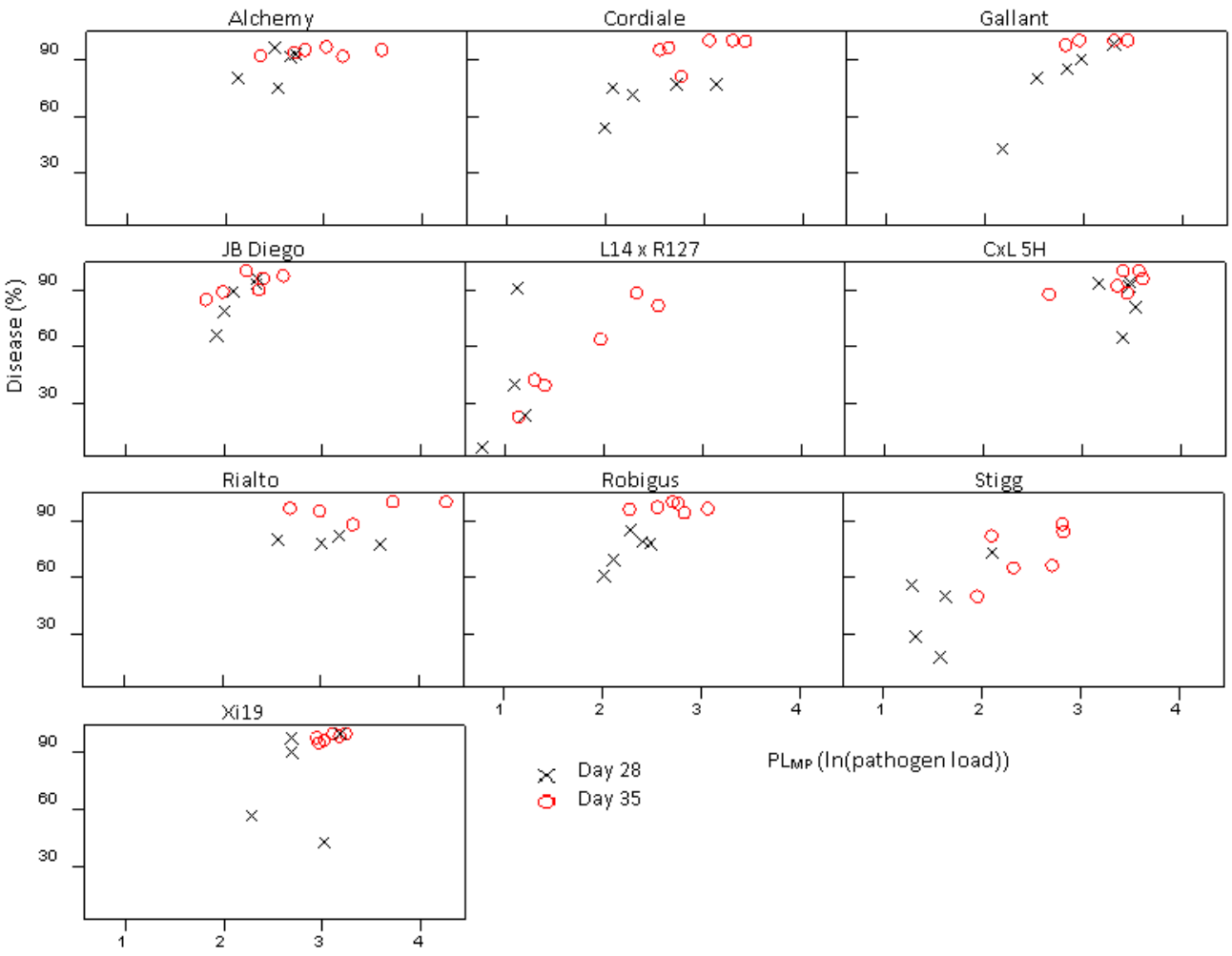


Fig 6.10. The relationships between disease symptoms and pathogen load in the 10 genotypes as standardised by multiplex qPCR. The two assessment dates (28 and 35 dpi) are shown separately.

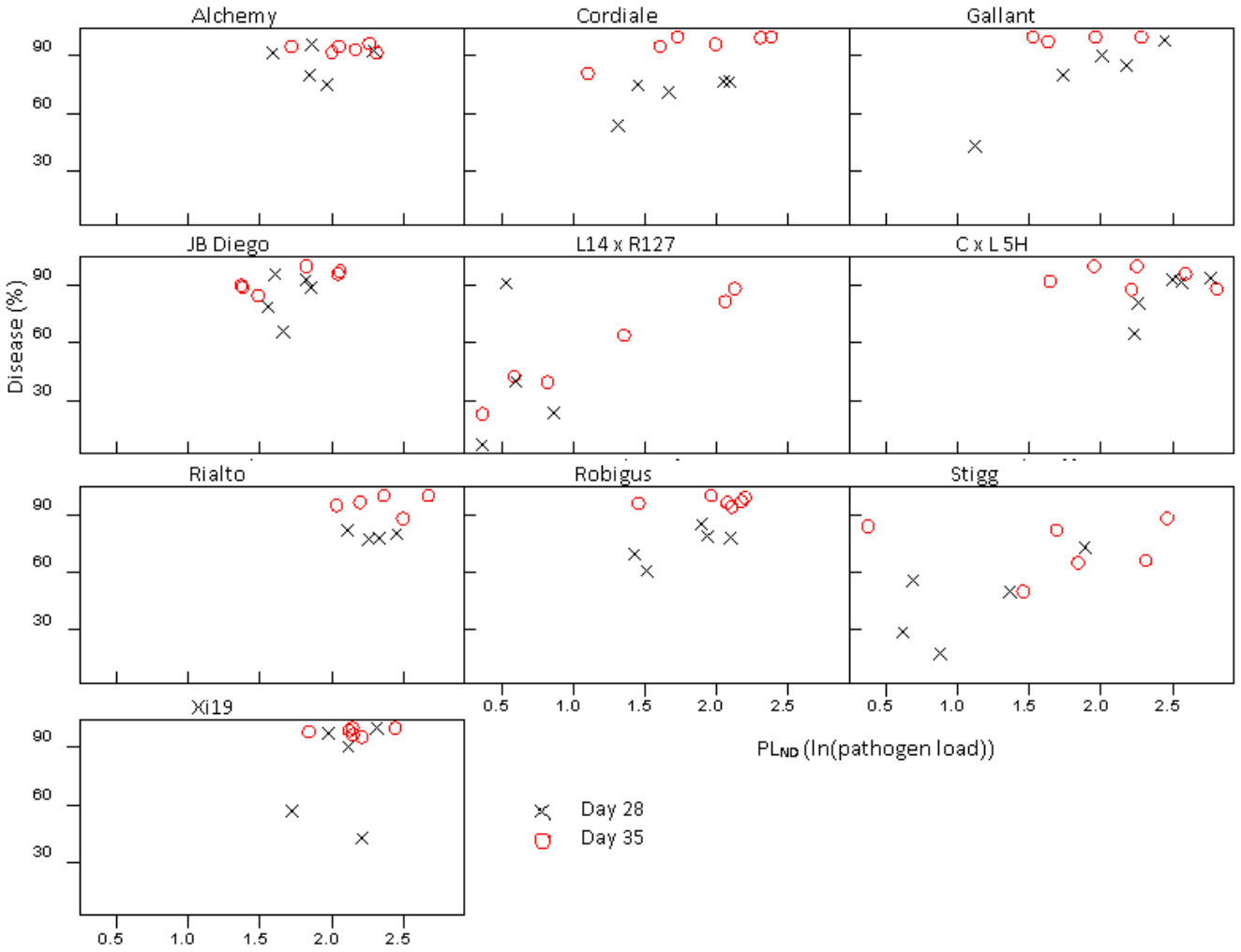


Fig 6.11. The relationships between disease symptoms and pathogen load in the 10 genotypes as standardised by total DNA quantified by NanoDrop. The two assessment dates (28 and 35 dpi) are shown separately.

In order to quantify the relationship between pathogen load (PL) and visual symptoms, “pathogen load per unit disease” was calculated for the multiplex qPCR (pg *Z. tritici* ng wheat^{-1} per disease %) and NanoDrop standardised DNA (pg *Z. tritici* ng total DNA^{-1} per disease %). There was no difference between sampling dates for either of the methods, but there were large differences between genotypes in both methods ($p<0.001$). The average PL_{MP} symptom^{-1} was 0.19 and average the PL_{ND} symptom^{-1} was 0.074 for the total DNA (table 6.8). The two genotypes displaying the lowest pathogen load per unit disease were JB Diego and L14xR127 for both methods. The Cadenza x Lynx 5H genotype had the highest pathogen load per unit disease for both methods, significantly higher than the other genotypes with the exception of Rialto (Table 6.8).

Table 6.8. Pathogen load per unit disease symptoms. The genotypes were analysed by a one-way ANOVA, and grouped according to Bonferroni multiple comparison tests.

Genotype	**Multiplex qPCR**		**NanoDrop**	
Alchemy	0.172	ab	0.0730	abcd
Cordiale	0.182	ab	0.0638	ab
Gallant	0.210	bc	0.0669	abc
JB Diego	0.0910	a	0.0516	ab
L14xR127	0.0912	a	0.0432	a
CxL 5H	0.326	d	0.112	d
Rialto	0.320	cd	0.107	cd
Robigus	0.134	ab	0.0675	abc
Stigg	0.133	ab	0.0658	ab
Xi19	0.218	bcd	0.0891	bcd
Mean	0.186		0.0737	
P genotype	<0.001		<0.001	
SED (df)	0.0347 (9)		0.0123 (9)	

6.4 Discussion

The qPCR assay targets used in this experiment were chosen from peer-reviewed publications (Bearchell et al., 2005, Ronning et al., 2006). The *Z. tritici* target was the β-tubulin gene, developed by Bearchell et al. (2005) to detect changes in the *Z. tritici* to *Stagonospora nodurum* ratio in historical wheat samples. Calibration curves for this target showed high efficiency (98%) and accuracy (r^2-value close to 1.0). There was no information about standard curves or dilution factors in the original publication, but the authors reported a limit of detection (LOD) value of 0.1 pg for *Z. tritici* (Bearchell et al., 2005). The LOD value in this study was higher at 1 pg but is still within the normal range used for pathogen detection in the literature; between 0.1 and 1 pg (Guo et al., 2006, Fredlund et al., 2008, Nicolaisen et al., 2009). The *Z. tritici* genome is 39.7 MB (Goodwin et al., 2011) which equates to 40 fg per genome. As the bioinformatics analysis indicated two binding sites per genome, which means that in the 25 genome copies (1 pg *Z. tritici* DNA) there are 50 potential targets. Another study performed by Selim et al. (2014) used this assay to study wheat varietal resistance, but had a standard curve in the range of 10^2 to 10^7 cloned gene copies. Even though 100 copies is considerably higher than the LOD determined by this study and the original publication, it was low enough to discover varietal differences in pathogen biomass (Selim et al., 2014).

The wheat assay was originally developed by Ronning et al. (2006) for amplifying wheat content in flour and other potentially GMO-containing products. The standard curve in our assay was based on Ronning et al. (2006) for comparison, with three-fold dilutions ranging between approximately 10,000 and 40 copies. The r^2 values for the standard curves were also similar (0.995 in the original compared to 0.998 in the present study) for the two (Ronning et al., 2006). The LOD-value of the original paper was 5 copies, much lower than the 28.5 copies in this study. However, as this study is quantifying DNA extracted from wheat leaf samples this limitation in detection is unlikely to be an issue. The gene targeted in this assay was annotated as a single-copy gene (Nelson et al., 1995, Ronning et al., 2006). However, a NCBI BLAST search indicated that the assay targeted two genes; the original target gene PKABA1 and a gene named W55c. It is at the moment unclear if these are two separate genes or the same gene annotated twice, as NCBI is an

open source database and W55c did not have any matches in the ENSEMBL database.

In order to account for variation in DNA extraction and starting materials, qPCR quantifications are nearly always standardised against a baseline value, commonly total DNA content in a sample (Fraaije et al., 2001, Nicolaisen et al., 2009). Very few qPCR assays have quantified host DNA along with pathogen DNA, and no *Z. tritici* - wheat multiplex qPCR assays have been published. One of the main aims of this chapter was to determine if it is more accurate to standardise *Z. tritici* by multiplex qPCR targeting both pathogen and host, or to total extracted DNA. All three standardisation methods were successfully able to differentiate between contrasting inoculum and to some extent between Rialto and Xi19 pathogen loads. There was a major issue with quantifying total DNA by gel electrophoresis; a number of samples were low concentration and/or low quality. In the second inoculation experiment, only 52 out of 72 samples were successfully quantified using this method which likely impacted on the statistical validity of the analysis. For this reason, the gel electrophoresis method was excluded from the following glasshouse experiment.

All three quantification methods were better at discriminating genotype differences in disease presence compared to assessments of visual disease symptoms, and all displayed strong relationships between disease severity and pathogen loads. There were fewer differences between the methods than expected, although PL_{MP} displayed higher sensitivity at very low disease levels in the inoculation experiments. The correlation between disease symptoms and pathogen loads were considerably weaker in the variety experiment for both of the methods included.

In the inoculum concentration experiments, Rialto consistently displayed stronger pathogen load – disease symptom relationships than Xi19. This did not appear to be a latency effect, as there was little difference in the timing of visible symptoms starting to appear. Xi19 seems to yield consistently less pathogen DNA than Rialto for high disease symptoms, which could be due to a partial resistance mechanism restraining the spread of in-leaf pathogen while allowing disease symptoms to appear. It has been shown by microscopy that resistant wheat genotypes contain less *Z. tritici* mycelia than susceptible genotypes (Kema et al., 1996a), but this was also reflected in the

amount of visible symptoms. Selim et al. (2014) used qPCR to detect differences in *Z. tritici* biomass in two wheat genotypes of contrasting resistance. This was however again corresponding to differences in latency periods and appearance of visible disease symptoms.

In order to explore the disease symptom-pathogen load relationships further, a subsequent glasshouse experiment was carried out with 8 additional genotypes of more contrasting genetic backgrounds. Considerably larger differences were seen when the number of varieties was expanded. The two genotypes with least visible symptoms were L14xR127 and Stigg (the most resistant commercial winter wheat recently available on the Irish market). Both of these had lower than average pathogen load per unit disease symptom (PL symptoms^{-1}), particularly L14xR127 which was one of the genotypes with the lowest score. In general, there was no agreement between PL symptoms^{-1} and resistance rating, even though the genotypes with resistance rating 6 and 7 were below the average (Fig 6.12; Table 6.12). A genotype of particular interest is JB Diego, a susceptible genotype that consistently displayed high disease symptoms but low pathogen loads. There was no difference between the PL symptoms^{-1} at 28 and 35 dpi, which indicates that the pathogen load-disease symptom relationship could be stable over a range of severities.

The two doubled-haploid genotypes L14xR127 and CxL 5H displayed very low and very high PL symptoms^{-1}, respectively. Both of these genotypes were previously screened for in-field disease tolerance. In 2012, L14xR127 displayed disease tolerance, and CxL 5H displayed intolerance. Rialto was also determined to be intolerant in 2012, and displayed high PL symptoms^{-1} in this experiment. Furthermore L14xR127 displayed a high seedling disease resistance in the glasshouse, and also little disease symptoms in the field season 2012 (although slightly higher symptoms in 2014) and was determined as disease tolerant. In opposite, CxL 5H and Rialto which displayed susceptibility in both glasshouse and the field experiment in 2012 were determined to be intolerant. It is possible that there is some association between disease tolerance and disease resistance, both for visual disease symptoms and for restriction of in-leaf pathogen growth. In order to determine the link between disease tolerance and disease resistance, pre-season glasshouse screens for disease resistance could be conducted to identify genetic variation in disease resistance as opposed to disease tolerance and disease escape.

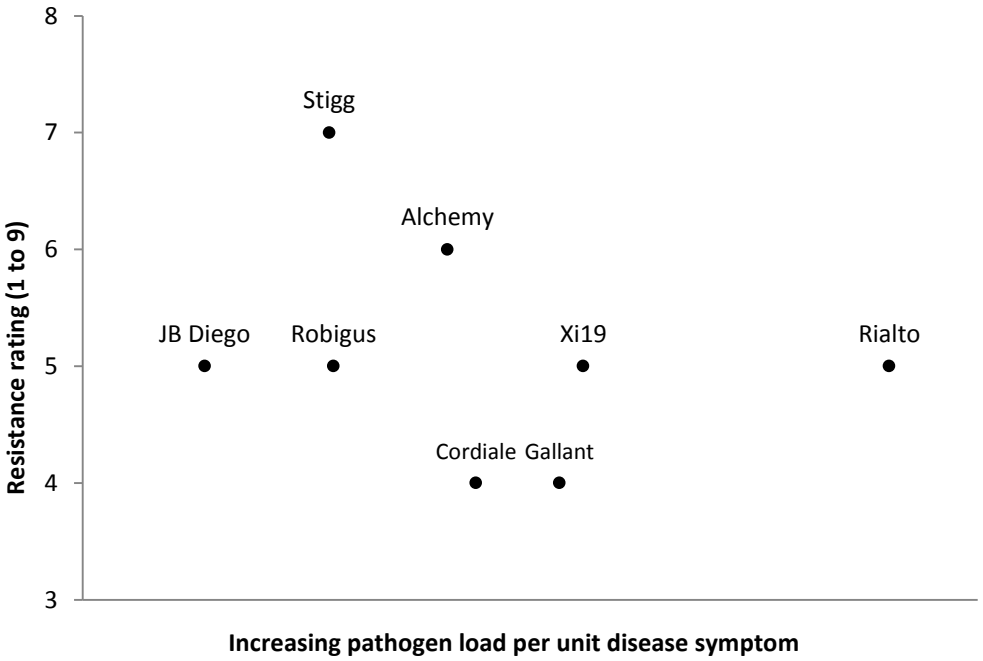


Fig 6.12. Illustrating the relationships between pathogen load per unit disease severity and the latest available STB resistance rating for each variety. The two double haploid genotypes are not included as they have no official resistance rating.

In addition to the contrasting amounts of *Z. tritici* biomass per unit disease between genotypes, there were also differences in slopes between *Z. tritici* biomass and disease symptoms. There is little literature available on the relationship between *Z. tritici* symptoms and pathogen biomass, and no general practise on how to fit the data sets. Guo et al. (2006) showed a strong non-linear relationship for a single wheat cultivar (Ritmo) between pathogen load and *Z. tritici* disease severity, but did not report which nonlinear function was used in the regression analysis. Gouache et al. (2011) used the same *Z. tritici* target as this study to quantify the mycelia growth – disease symptom relationship, and found a linear relationship between thermal time and *Z. tritici* copies over a range of genotypes. The same study found a non-linear relationship between PL symptoms^{-1} and thermal time, but did not report a regression analysis for the pathogen load-disease symptom relationship. Fraaije et al. (2001) found a reasonably strong linear relationship between lesion number and pathogen load using a PCR/SYBR green assay, over a range of genotypes.

In this study, linear regression was used for the regression analyses. However, looking at the distribution of the pathogen load – symptoms relationships for the individual genotypes it is apparent that not all genotypes will have the best fit to the same curve (Fig 6.10-11). Some of the genotypes had more linear relationship between pathogen loads and symptoms; for instance L14xR127 and JB Diego. In contrast, Gallant and Robigus had a better fit to quadratic functions (Fig 6.13). Several genotypes in the variety experiment displayed no relationship between pathogen load and symptoms regardless of the method of data analysis; among these were CxL 5H and Rialto (Fig 6.10-11).

It is well known that wheat varieties will differ in disease severity (disease resistance) and development (latency and partial resistance), but there is no literature suggesting differences in the amount of in-leaf pathogen to disease symptoms. This could possibly in part explain variations in the visible disease symptoms and grain yield relationship. Restricting in-leaf pathogen load could be a potential trade-off cost affecting the final grain yield, but it could also mean that less leaf assimilates would be lost to the pathogen. In the next chapter (7), the relationship between in-field pathogen load, disease symptoms and grain yield is investigated.

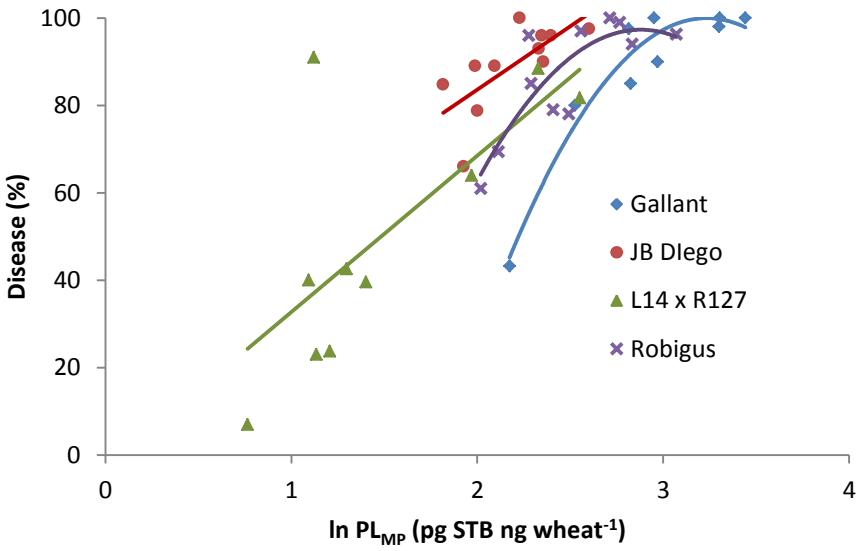


Fig 6.13. Examples of relationships between pathogen loads (quantified by multiplex qPCR) and disease symptoms for four genotypes. A linear function was the best fit for L14xR127 and JB Diego, while Robigus and Gallant displayed quadratic distributions.

6.5 Conclusions

There were fewer differences between quantifying pathogen loads by different standardisation methods than expected, and there was little difference between the multiplex PCR and NanoDrop (total DNA) methods. However, agarose gel electrophoresis proved unsuitable due to the inability to visualise the low quality DNA that often accompanies highly diseased samples. The overall relationships between pathogen loads and disease symptoms were strong in all glasshouse experiments, and different levels of initial inoculum could be differentiated which indicates sensitivity in the assays. However, there were differences between genotypes in the pathogen load-disease severity relationships, indicating that it might not be possible to accurately relate pathogen loads to disease severity over a wide range of genotypes using a single equation. The differences in expressed symptoms and pathogen biomass have not been previously shown in *Z. tritici* – wheat interactions, and future studies will investigate the relationship of this to yield potential and apparent disease tolerance.

7. Pathogen loads, disease symptoms and grain yield in field experiments

7.1 Introduction

Disease tolerance can be quantified in a number of ways, as the overall definition is maintaining performance in the presence of expressed disease (Ney et al., 2013). Research performed on non-graminaceous species could specify performance as plant biomass, but for this thesis grain yield is used to define plant fitness. However, the method of quantifying disease presence can be nearly as variable as the fitness traits. Disease can be quantified in a number of ways; for example symptom area, number of lesions and green area loss (Paveley, 1999, Nutter et al., 2006). These can in turn be expressed as a single measure or integrated over time (Bryson et al., 1997). For instance, Parker et al. (2004) quantified disease by healthy area duration and Inglese and Paul (2006) studied disease tolerance using AUDPC as a disease measure.

Disease tolerance is essentially measures of the disease-yield relationship over a range of varieties. Although attempts to standardise this relationship with a single equation have been done, most studies show substantial site-season variations in this relationship in addition to the host genotype variation (Paveley, 1999). In order to minimise site-season effects, healthy area duration (an integrated measure of green area over time) can be used in disease tolerance studies (Bingham et al., 2009). Using remaining photosynthetic green area as a measure of disease in disease-yield relationships is generally considered more accurate, as grain yield loss caused by leaf pathogens is mainly due to restriction of green leaf area (Paveley, 1999). There can at times be considerable differences between genotype rankings for the percentage of visual disease symptoms and green leaf area percentage. It is currently unknown to what extent the non-lesion green area loss is caused by in-leaf pathogen presence or physiological costs of restricting pathogen growth.

The overall aim of this chapter is to quantify the impact of in-leaf disease presence on visual disease symptom development, non-lesion green area loss, and final grain yield. To do so, *Zymoseptoria tritici* DNA was quantified in

flag leaf samples collected from field experiments in 2012 and 2014 and compared to visual disease measurements and grain yield variation.

The main hypotheses tested in this chapter are:

1. There will be differences in the actual pathogen load between wheat genotypes.
2. The ratio between symptom expression and pathogen load will differ between wheat genotypes.
3. A high pathogen load per unit visual symptoms will have an increased impact on yield loss and cause increasing non-lesion green leaf area loss.
4. There will be a relationship between the visual symptom – pathogen load ratio and disease tolerance.
5. Disease tolerance calculated by pathogen load will differ from disease tolerance calculated by AUDPC and HAD, and there will be differences in in which physiological traits affect disease tolerance calculated from pathogen loads, as compared to HAD measurements.

7.2 Materials and Methods

Flag leaf samples from the 49 genotypes in the 2012 field experiment and the 16 genotypes in the 2014 field experiment were collected and assessed for disease symptoms. The flag leaves in 2012 were collected as 0.5 cm^2 leaf cores, which were sampled evenly along the leaves in three places on each leaf, and whole flag leaves were sampled in 2014. DNA extractions were carried out using CTAB extraction for all leaf samples, with the exception of the GS59+14d sampling in 2012 which was extracted using a spin-column kit. Both of these methods are detailed in Chapter 3 (Materials and Methods). The *Z. tritici* DNA in these samples was quantified and standardised against wheat DNA in the multiplex qPCR assay developed in Chapter 6.

Assessments of disease symptoms and green leaf area percentage took place at GS59, GS59+14d and GS59+28d in both field experiments. In 2012, the same leaves assessed for disease were sampled for molecular analysis. In 2014, however, the main disease assessments were carried out on random tillers in each experimental plot, and flag-leaf samples were collected at GS59+14d and GS59+28d. A lab-based disease assessment took place at GS59+14d on the flag-leaves collected for molecular analysis.

All statistical analyses were performed on GenStat 14th ed. Differences between treatments and genotypes were primarily analysed by one-way ANOVAs. An analysis of covariance was used in order to analyse the relationships, by first determining the overall relationship by fitting a single line to the full data set. Second, the regression intercept is allowed to vary for each genotype (i.e. a parallel regression model) and finally both regression intercepts and slopes are allowed to vary for each genotype.

7.3 Results

7.3.1 Quantifying pathogen loads in field samples

The pathogen loads at three assessments in the 2012 field experiment (GS59; +14d and +28d) and two assessments in 2014 (GS59+14d and +28d) were quantified for both fungicide treatments. As in Chapter 6, pathogen load is defined as the ratio between STB DNA and wheat DNA as quantified by multiplex qPCR. The pathogen load will be referred to as PL throughout the chapter and the units of PL will be referred to as pg (even though it is actually log pg STB ng wheat^{-1}) for the text for concision.

At ear emergence in 2012, there was a slight decrease in overall PL in the fungicide protected treatment at 0.005 pg compared to 0.029 pg in the untreated treatment ($p<0.001$; Table 7.1). There were also differences in pathogen load between genotypes in the untreated samples ($p=0.005$) but not in the protected samples. In the untreated treatment pathogen loads varied between 0.0011 and 0.195 pg and between 0 and 0.061 pg in the protected treatment.

At the second assessment in 2012 (GS59 + 14d), the application of fungicides decreased the average PL from 0.272 to 0.056 pg ($p<0.001$; Table 7.1). There were differences in pathogen load between genotypes, which ranged from 0.080 to 1.30 pg when untreated ($p<0.001$) and 0.025 to 0.113 pg with fungicide protection (non-significant).

Unlike the previous two assessments only a subset of samples from GS59+28d were analysed, 151 samples in total, due to extreme flag leaf disease causing senescence in a large number of samples. At this assessment there were differences between the fungicide treatments ($p<0.001$) at 0.416 and 0.031pg, respectively (Table 7.1). As seen in the

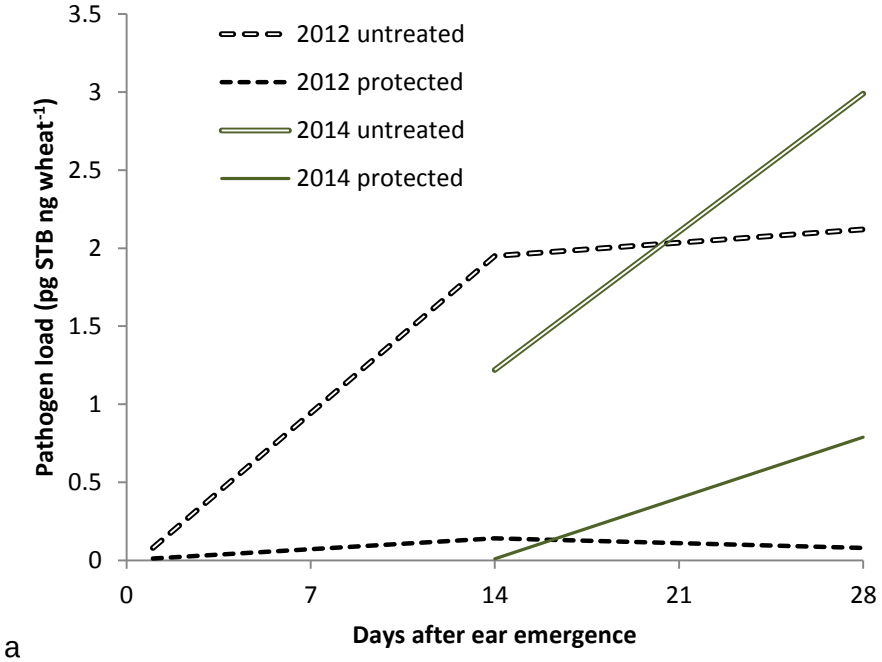
previous assessments, there were genotype differences in the untreated samples ($p=0.013$) but not the protected. In the untreated samples, the genotypes ranged 0.061 – 1.08 pg and in the protected treatment the range was 0.010 - 0.184 pg.

In 2014, there was a significant difference between fungicide treatments ($p<0.001$) and genotypes ($p<0.001$) at GS59+14d, in which the average untreated pathogen load was 0.255 pg (ranging 0.025-0.694) and the fungicide protected average was 0.004 pg (ranging 0.00 to 0.016; Table 7.2).

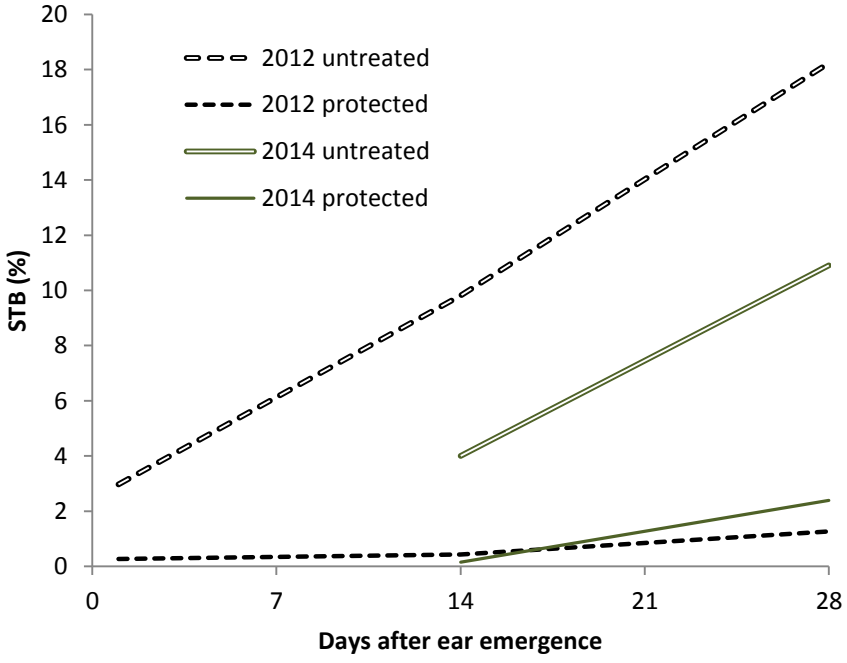
At GS59+28d in 2014, there were also a decreased number of samples analysed due to high disease presence; 81 samples out of 96 were analysed. There was an overall difference between the fungicide treatments, the fungicide protected average was 0.155 pg compared to 0.482 pg in the untreated treatment ($p<0.001$; Table 7.2). There were differences between genotypes ($p<0.001$), in the untreated where the genotypes ranged 0.091 – 1.23 pg and in the protected treatment where genotypes ranged 0.008 – 0.693 pg.

In 2012, the overall pathogen load increased with time in the untreated treatment; starting from very little at ear emergence, increasing strongly at GS59 + 14d and finally there was a slight increase for GS59+28d (Table 7.1; Fig 7.1a). The pathogen loads seemed to plateau between GS59+14d and GS59+28d, but this was not reciprocated in the visual symptoms which increased nearly linearly (Fig 7.1a-b). In the protected treatment, the pathogen load increased from GS59 to GS59+14d, but then decreased slightly at GS59+28d (Table 7.1; Fig 7.1a). In 2014, the pathogen load increased strongly between GS59+14d and GS59+28d in both treatments (Table 7.2; Fig 7.1a). In general, the increase in disease symptoms seem to correspond better to the increase in pathogen load in 2014 compared to 2012 (Fig 7.1a-b).

In order to normalise the pathogen load data, a logarithm (base 10) was used to transform the data. For the remaining analyses in this chapter, the log-transformed pathogen load data were used.



a



b

Fig 7.1. Disease development over time in 2012 and 2014 field experiments. a) Pathogen load development over time for each fungicide treatment average; b) Disease symptom (%STB) development over time for each fungicide treatment average.

Table 7.1 Pathogen load (log pg STB DNA ng wheat DNA^{-1}) in 2012. At GS59, GS59+ 14 days and GS59+28 days.

	GS59		+14d		+28d	
Genotype	**Untreated**	**Protected**	**Untreated**	**Protected**	**Untreated**	**Protected**
CxL 14B	0.0012	0.0007	0.1120	0.0522	0.0855	*
CxL 3D	0.0033	0.0026	0.1076	0.0633	0.4901	*
CxL 41F	0.0171	0.0286	0.1172	0.0900	0.8334	*
CxL 42C	0.0139	0.0015	0.1655	0.0464	0.3840	*
CxL 43A	0.0093	0.0011	0.1076	0.0306	0.5939	0.0205
CxL 48D	0.0033	0.0012	0.1280	0.0379	0.3378	*
CxL 5H	0.0050	0.0083	0.1621	0.0675	0.4353	*
CxL 6E	0.0176	0.0003	0.2016	0.0694	0.5309	*
CxL 7a	0.0056	0.0039	0.1527	0.0601	*	*
CxL 8I	0.0018	0.0161	0.2666	0.0537	0.4426	*
L14 x R 10	0.1952	0.0024	1.2986	0.0383	*	*
L14 x R 104	0.0141	0.0014	0.3587	0.0612	0.5655	0.0506
L14 x R 107	0.0272	0.0041	0.1498	0.0711	0.5876	*
L14 x R 112	0.0527	0.0050	0.2055	0.0613	0.2476	0.0395
L14 x R 114	0.0011	0.0004	0.0868	0.0390	0.2316	0.0143
L14 x R 119	0.0222	0.0000	0.1813	0.0902	0.3425	0.0279
L14 x R 121	0.0336	0.0015	0.0803	0.0506	0.0614	0.0197
L14 x R 123	0.0163	0.0000	0.1726	0.0892	0.3178	0.0376
L14 x R 127	0.0066	0.0007	0.1293	0.0804	0.3380	0.0111
L14 x R 129	0.0062	0.0018	0.1468	0.0642	0.3008	0.0249
L14 x R 131	0.0391	0.0004	0.2127	0.0775	0.4683	*
L14 x R 133	0.0042	0.0003	0.1751	0.0430	0.3255	*
L14 x R 136	0.0181	0.0062	0.3768	0.0639	*	*
L14 x R 138	0.0352	0.0031	0.1768	0.0562	0.4560	0.0245
L14 x R 15	0.0537	0.0050	0.2168	0.0466	0.3772	0.0187
L14 x R 16	0.0132	0.0032	0.6210	0.0410	0.6992	*
L14 x R 19	0.0311	0.0035	0.5918	0.0690	0.4193	*
L14 x R 20	0.0123	0.0039	0.0905	0.0449	0.5624	0.0253
L14 x R 22	0.0048	0.0019	0.6826	0.0349	*	*
L14 x R 23	0.0225	0.0006	0.5067	0.0389	0.5420	*
L14 x R 35	0.0390	0.0191	0.3339	0.0426	0.1768	*
L14 x R 43	0.0509	0.0045	0.2319	0.0511	0.5589	0.0230
L14 x R 5	0.0716	0.0056	0.1706	0.1112	0.3643	0.0470
L14 x R 50	0.0324	0.0035	0.3443	0.0370	0.3995	0.0108
L14 x R 55	0.0227	0.0014	0.4907	0.0310	0.3895	*
L14 x R 59	0.0099	0.0014	0.1564	0.0253	0.1748	0.0144
L14 x R 62	0.0425	0.0010	0.4098	0.0341	0.2141	0.0319
L14 x R 66	0.0119	0.0052	0.1916	0.0344	0.3842	*
L14 x R 68	0.0221	0.0045	0.1937	0.0396	0.5777	0.0222
L14 x R 7	0.0345	0.0014	0.3889	0.1126	0.5463	0.0406
L14 x R 70	0.0345	0.0158	0.1443	0.0298	0.6475	*
L14 x R 74	0.0143	0.0011	0.1359	0.0382	0.3776	0.0272
L14 x R 80	0.0341	0.0000	0.1402	0.0410	0.1809	0.0103
L14 x R 82	0.0754	0.0607	0.2116	0.0629	1.0801	*
L14 x R 85	0.0637	0.0000	0.4186	0.0455	0.7340	*
L14 x R 89	0.0039	0.0060	0.2345	0.0667	0.5910	0.0342
L14 x R 91	0.0839	0.0009	0.7198	0.1117	0.5835	*
L14 x R 92	0.0354	0.0000	0.1788	0.0737	0.1421	0.0199
Rialto	0.0159	0.0037	0.1122	0.0542	0.9692	0.1840
Average	0.0294	0.0050	0.2717	0.0563	0.4161	0.0305
P G	0.005	NS	<0.001	NS	0.013	NS
SED (df)	0.0345 (85)		0.215 (94)		0.228 (42)	
P FT	<0.001	*	<0.001	*	<0.001	*
SED (df)	0.00405 (1)		0.0256 (1)		0.0310 (1)	

Table 7.2. Pathogen load (log pg STB ng wheat^{-1}) in 2014 at GS59+14 days and GS59+28 days.

	+14d		+28d	
Genotype	**Untreated**	**Protected**	**Untreated**	**Protected**
AxC 117	0.295	0.004	0.576	0.062
AxC 21	0.195	0.002	0.709	0.062
CxL 14B	0.053	0.002	0.095	0.008
CxL 43A	0.448	0.002	0.564	0.084
CxL 5H	0.089	0.007	0.482	0.102
CxL 7A	0.648	0.001	*	0.101
L14xR 114	0.025	0.003	0.193	0.021
L14xR 127	0.082	0.004	1.024	*
L14xR 133	0.091	0.003	0.477	0.250
L14xR 16	0.405	0.000	0.892	0.108
L14xR 19	0.694	0.016	0.924	0.693
L14xR 20	0.252	0.001	0.527	0.096
L14xR 22	0.653	0.000	1.227	0.626
L14xR 92	0.030	0.003	0.131	0.028
L8xR 10	0.070	0.011	0.091	0.012
L8xR 86	0.044	0.012	0.365	0.014
Average	0.255	0.004	0.482	0.155
P G	<0.001	NS	<0.001	<0.001
SED (df)	0.1217 (32)		0.1306 (23)	0.1046 (28)
P FT	<0.001	*	<0.001	*
SED (df)	0.04729 (1)		0.06170 (1)	

7.3.2 The relationships between pathogen load and disease symptoms

7.3.2.1 The relationship between pathogen load and disease symptoms at each assessment

The relationships between visual disease symptoms (presented in chapter 4) and pathogen load were determined for the three assessments in the 2012 field experiment and two assessments in 2014 by an analysis of covariance.

In 2012, the same leaves used to determine visual disease symptoms were used to determine pathogen load at all assessments. At ear emergence (GS59) the quantified in-leaf pathogen load accounted for 23.1% of the variation in disease symptom expression ($p<0.001$; Fig 7.2a). When each genotype regression intercept and slope were allowed to vary, the variation in disease symptoms accounted for by pathogen load increased to 38.2% ($p<0.001$). In the following assessment (GS59+14d), there was an overall relationship between pathogen load and disease symptoms accounting for 42.1% of the variation in disease symptoms ($p<0.001$; Fig 7.2b). When the regression slopes and intercepts were allowed to vary for each genotype, the pathogen load accounted for 55.3% of symptom variation ($p<0.001$). At the final assessment, GS59 + 28d, there was an strong overall relationship

between pathogen load and disease symptoms accounting for 81.0% of the variation ($p<0.001$; Fig 7.2c). This relationship increased further when each genotype regression slope and intercept was allowed to vary, with pathogen load accounting for 89.4% of the symptom variation ($p<0.001$).

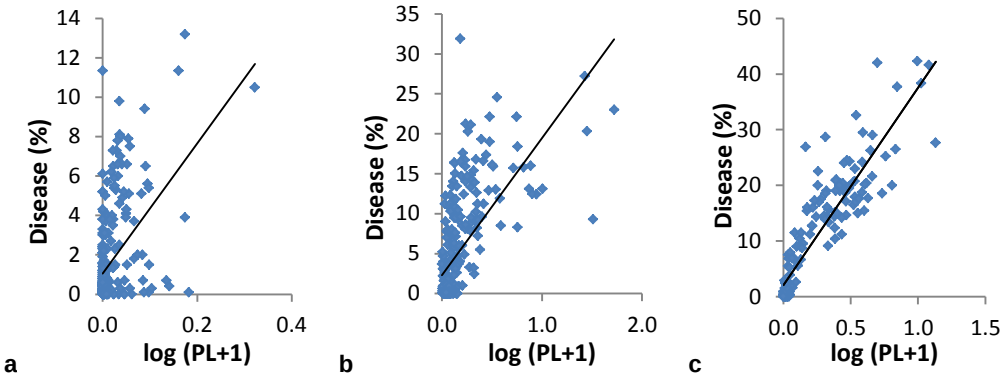


Fig 7.2. The relationships between pathogen load (PL) and visual disease symptoms in 2012. Each disease assessment is showed separately; a) GS59; b) GS59+14d; and c) GS59+28d.

In 2014, the same leaves that were quantified for pathogen loads were also assessed for visual disease symptoms at GS59+14d but not at GS59+28d. For this section this assessment will be referred to as “lab-based”. In-field assessments were performed on GS59+14d and GS59+28d, and these assessments were also used to calculate AUDPC.

At GS59+14d in 2014, the pathogen load accounted for 77.7% of the disease symptom variation in the lab-based disease assessment ($p<0.001$; Fig 7.3a). When genotype regression slopes and intercepts were allow to vary, the pathogen load accounted for 91.0% of symptom variation. At the same assessment date, pathogen load accounted for only 34.8% of symptom variation in the in-field disease assessment ($p<0.001$; Fig 7.3b). This increased to 56.3% ($p=0.015$) when genotype intercepts were allowed to vary, but there was no significant increase in varying genotype regression slopes. At GS59+28d, there was an overall relationship between disease symptoms and pathogen load ($p<0.001$; Fig 7.3c), in which pathogen load accounted for 56.0% of variation in disease symptoms. There was no genotype interaction or group effect in this relationship.

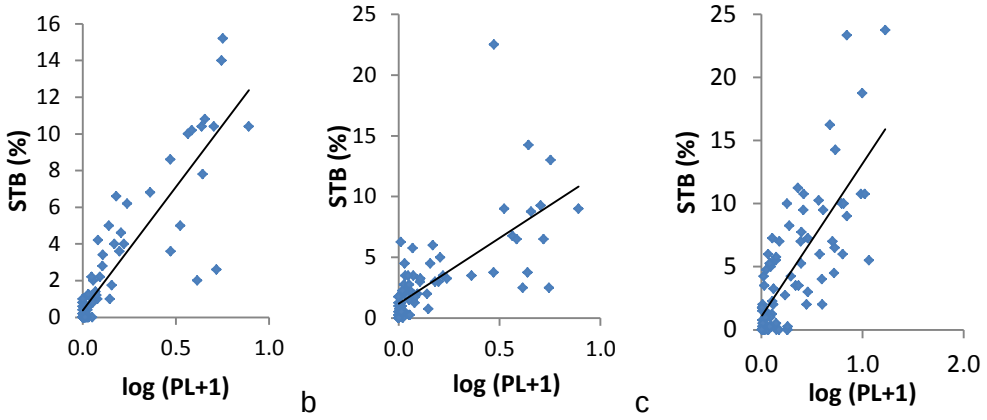


Fig 7.3. The relationships between visual STB symptoms and pathogen load for a) the lab-based disease assessment at GS59+14d; b) the in-field disease assessment at GS59+14d; and c) the in-field assessment at GS59+28d.

7.3.2.2 The relationship between pathogen load and AUDPC

Disease symptoms can be integrated over time using an area under the disease progress curve (AUDPC). The relationships between the quantified pathogen loads and AUDPC (calculated from visual disease symptoms as percentages) were determined for the two years, using analysis of covariance.

As mentioned in Chapter 4, the AUDPC of all plots could not be determined over all three assessments in 2012. Because of this, the relationship between pathogen load at GS59 and AUDPC calculated between GS59 and GS59+14d was analysed as it contained the full data set. In this relationship, pathogen load accounted for 17.9% of variation in AUDPC ($p<0.001$; Fig 7.4a). This increased slightly to 32.6% when genotype regression intercepts and slopes were allowed to vary ($p=0.003$). Pathogen load at ear emergence accounted for 10.8% ($p<0.001$; Fig 7.4b) of variation in AUDPC calculated over three assessments (in total 142 plots could be included in the analysis). There was no genotype covariate interaction or group effect in this relationship.

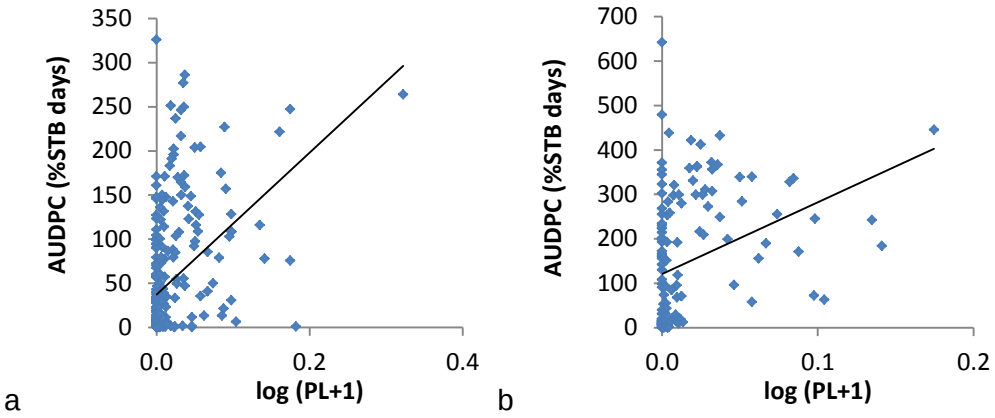


Fig 7.4. The relationships between pathogen loads at GS59 in 2012 and a) AUDPC calculated between GS59 and +14d; b) AUDPC calculated over three assessments.

The pathogen load at the second assessment (GS59+14d) was also analysed with AUDPC calculated over the first two assessments; GS59 and GS59+14d. Overall, the pathogen load accounted for 38.1% of the variation in AUDPC ($p<0.001$; Fig 7.5a). There was a genotype interaction in this relationship, as when the intercepts and slopes of each genotype regression were allowed to vary the relationship increased to account for 62.2% of the variation ($p<0.001$).

The pathogen load at GS59+14d accounted for 12.3% of the variation in AUDPC calculated between 59+14d and 59+28d ($p<0.001$; Fig 7.5b). This increased to 42.0% when varying genotype regression intercepts, displaying a genotype effect ($p<0.001$), but there was no genotype interaction in this relationship.

The pathogen load at GS59+14d accounted for 33.3% of variation in the AUDPC calculated over three assessments ($p<0.001$; Fig 7.5c). This increased to 46.2% ($p=0.008$) when the genotype regression intercepts were allowed to vary independently. There was no increase when varying the genotype regression slopes, and hence no genotype interaction.

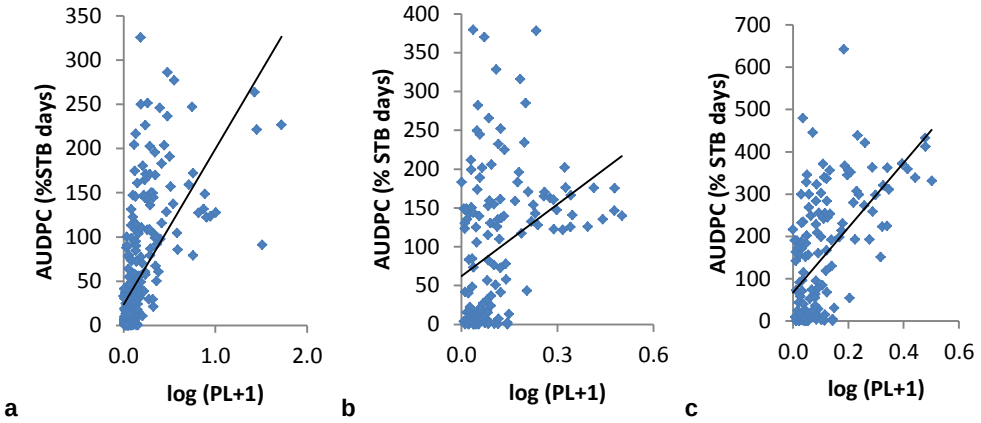


Fig 7.5. The relationships between pathogen loads at GS59+14d in 2012 and a) AUDPC calculated between GS59 and GS59+14d; b) AUDPC calculated between GS59+14d and GS59 +28d; and c) AUDPC calculated over all three assessments.

At the final assessment in 2012 (GS59+28d), the relationship between pathogen load and AUDPC calculated between GS59+14 and GS59+28d was also investigated. The overall relationship was strong, with pathogen load accounting 79.9% of the variation ($p<0.001$; Fig 7.6a). This increased to 89.2% when both slopes and intercepts of each genotype regression was allowed to vary, hence displaying a strong genotype interaction ($p<0.001$).

The relationship between pathogen load at GS59+28d and AUDPC, calculated over all three assessments, was determined. Overall, pathogen load accounted for 65.1% of the variation ($p<0.001$; Fig 7.6b). There was a genotype effect as the relationship increased to 70.2% when intercepts were allowed to vary ($p=0.041$), but there was no genotype interaction in this relationship.

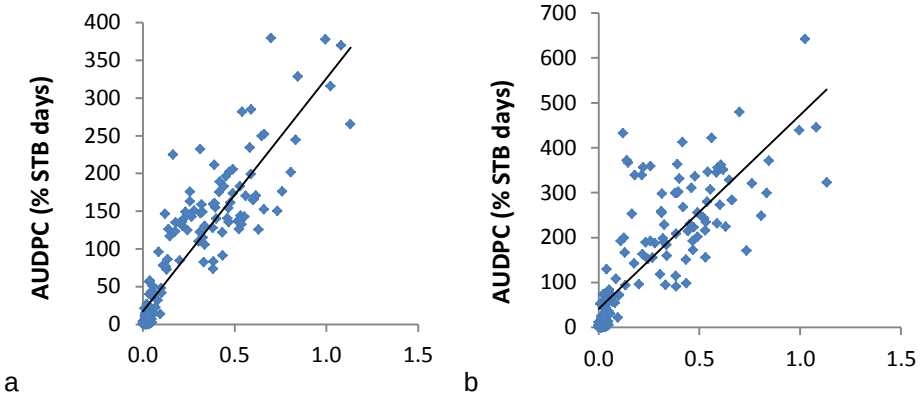


Fig 7.6. The relationships between pathogen loads at GS59+28d in 2012 and a) AUDPC calculated between GS59+14d and GS59+28d; b) AUDPC calculated over all three assessments.

In 2014, as in 2012, AUDPC was calculated between GS59 and GS59+14d, GS59+14d and GS59+28d, and for three assessments (GS59, +14d and +28d). The pathogen load at GS59+14d displayed reasonably strong relationships with all of these. The pathogen load accounted for 48.3% of the variation in AUDPC calculated between GS59 and GS59+14d ($p<0.001$; Fig 7.7a). The relationships between pathogen load and AUDPC calculated between GS59+14d and GS59+28d and between pathogen load and AUDPC calculated over all three assessments were similar at 49.0% ($p<0.001$) and 50.2% ($p<0.001$) respectively (Fig 7.7b-c). There was no genotype effect or interactions in any of these relationships.

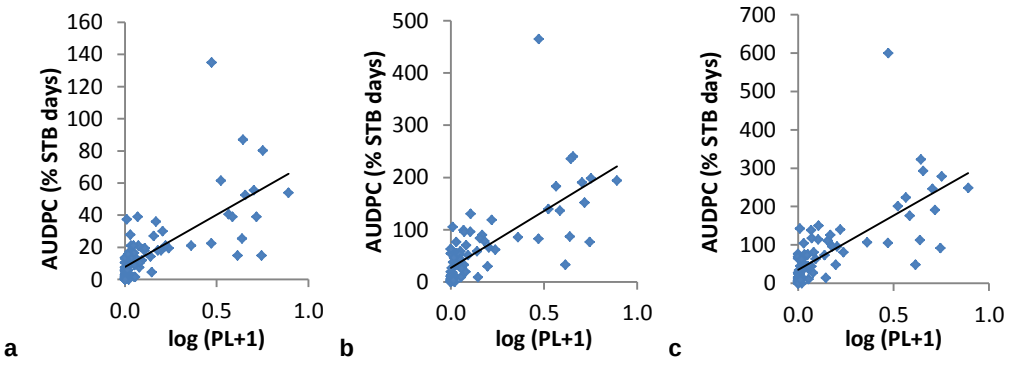
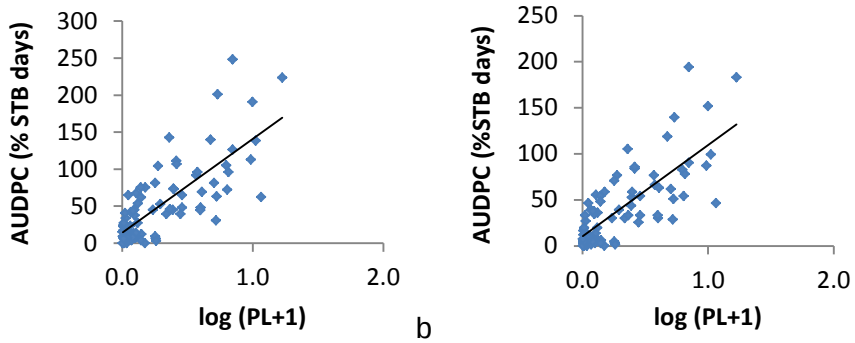


Fig 7.7. Relationships between pathogen loads quantified at GS59+14d in 2014 and AUDPC calculated between a) GS59 and GS59+14d; b) GS59+14 d and GS59 + 28d; and c) GS59, GS59+14d and GS59+28d.

At the final assessment in 2014 (GS59+28d), the overall relationship between pathogen load and AUDPC calculated between GS59+14d and GS59+28d accounted for 58.0% of variation ($p<0.001$; Fig 7.8a). The relationship between pathogen load and AUDPC calculated over all three assessments was very similar, with pathogen load accounting for 57.9% of

AUDPC variation (p<0.001; Fig 7.8b). There was no genotype effect or interaction in any of these relationships.



a b

Fig 7.8. Relationships between pathogen load at GS59+28d and a) AUDPC calculated between GS59+14d and GS59+28d and b) AUDPC calculated over three assessments.

7.3.2.3 Integrated pathogen load and its relationship to AUDPC

AUDPC is an integrated measure of disease progress over time. In order to compare the pathogen load progression, pathogen load integrated over time was calculated by the same formula by replacing the visual disease scores with pathogen load (pg STB ng wheat^{-1}). This integrated pathogen load will be abbreviated to iPL for the remainder of the chapter. All iPL values used in the analyses below were transformed using a base 10 logarithm.

In 2012, when AUDPC and iPL were both calculated between GS59 and GS59+14d, there was an overall relationship between these as iPL accounted for 56.4% of variation in AUDPC (p<0.001; Fig 7.9a). When both genotype regression slopes and intercepts were allowed to vary the relationship accounted for 71.2%, displaying a significant genotype interaction (p<0.001).

The iPL calculated between GS59+14d and GS59+28d had an overall relationship with AUDPC calculated over the same two assessments, accounting for 85.1% of the variation (p<0.001; Fig 7.9b). There was a strong genotype effect for this relationship, as when genotype regression intercepts were allowed to vary the relationship increased to account for 90.0% (p<0.001). There was also a genotype interaction in this relationship; varying the genotype regression slopes increased the relationship to 92.2% (p=0.004).

Finally, in 2012 there was also a strong relationship between iPL and AUDPC when both were calculated over all three assessments. iPL accounted

for 74.9% of the variation in AUDPC (p<0.001; Fig 7.9c), which improved to 87.1% when genotype regression intercepts and slopes were allowed to vary (p<0.001).

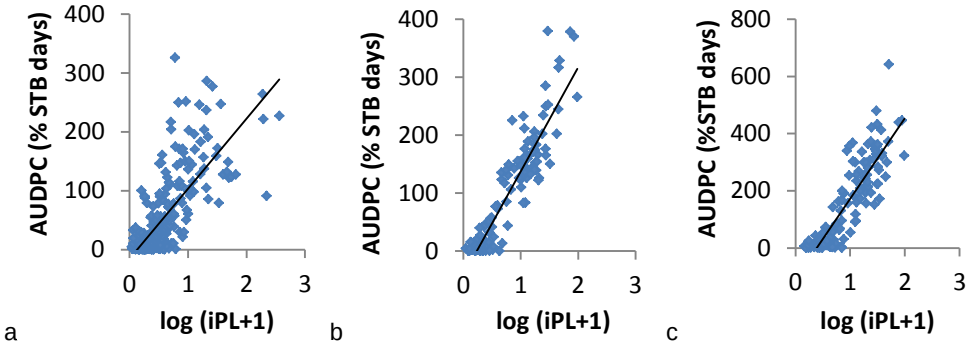


Fig 7.9. The relationships between AUDPC (% STB days) and iPL (pg STB ng wheat^{-1} days). a) Both AUDPC and iPL calculated between GS59 and GS59+14d; b) both factors calculated between GS59+14d and GS59+28d; c) both factors calculated over all three assessments.

In 2014, iPL was calculated between GS59+14d and GS59+28d. There was an overall relationship between iPL and AUDPC calculated between GS59+14d and GS59+28d accounting for 61.9% of AUDPC variation (p<0.001; Fig 7.11a). The overall relationship between iPL and AUDPC calculated over three assessments was very similar as iPL accounted for 61.5% of AUDPC variation (p<0.001; Fig 7.11b). Although there was a near-significant genotype interaction in the iPL – AUDPC (calculated over three assessments) relationship (p=0.097), there were no significant genotype effects or interactions in these relationships.

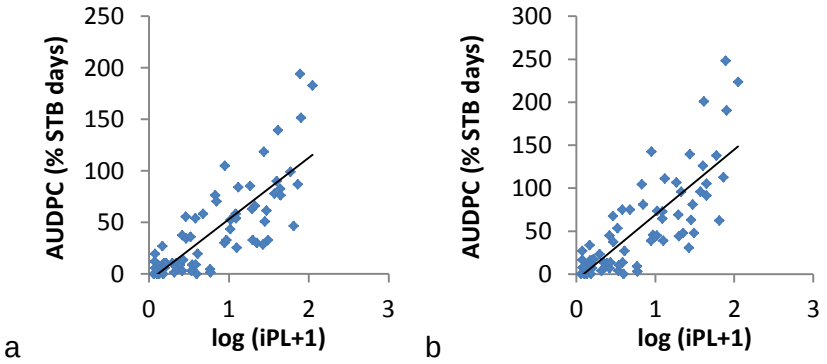


Fig 7.10. The relationships between AUDPC (% STB days) and iPL (pg STB ng wheat-1 days) in 2014. a) AUDPC and iPL calculated between GS59+14d and GS59+28d; b) AUDPC and iPL calculated over three assessments.

7.3.2.4 Predicting future symptoms?

In order to determine if the quantified pathogen loads could be used to predict future disease symptoms, the pathogen load at GS59 and GS59+14d was compared to the disease symptoms at the following assessments.

In 2012, the pathogen load at GS59 had an overall relationship with the disease symptoms at GS59+14d accounting for 17.9% of symptom variation ($p<0.001$). There was no genotype effect when varying genotype regression intercepts, but there was a near-significant genotype interaction in this relationship which increased the relationship to account for 23.1% ($p=0.055$) when genotype regression intercepts and slopes varied.

The pathogen load at GS59+14d in 2012 had an overall relationship with disease symptoms at GS59+28d accounting for 24.1% ($p<0.001$). There was no genotype effect in this relationship, but when both genotype regression intercepts and slopes were allowed to vary the symptoms variation accounted for increased to 40.5% ($p<0.001$).

In 2014, the pathogen load at GS59+14d had an overall relationship with disease symptoms at GS59+28d accounting for 47.1% of symptom variation ($p<0.001$). There was no genotype effect or interaction in this relationship.

7.3.3 Genotype differences in the amount of pathogen load per unit visual disease symptoms

In order to determine if there were genotype differences between the visual disease expression and the actual in-leaf pathogen load, the pathogen loads per unit visual disease (abbreviated as PL symptoms^{-1} in Chapter 6) were calculated for each genotype in the untreated treatment.

There was no significant difference between the assessments in PL symptoms^{-1} in 2012, even though the average pathogen loads varied from 0.082 pg STB %$^{-1}$ at GS59 to 0.158 pg STB %$^{-1}$ at GS59+14d and finally to 0.101 pg STB %$^{-1}$ at GS59+28d ($p=0.102$; Fig 7.12a). When all three assessments were combined there was no significant difference between genotypes, and no timing x genotype interaction. There were no genotype differences in the two first assessments in 2012 when each assessment was analysed separately. There were, however, significant genotype differences in PL symptoms^{-1} in the final assessment ($p=0.002$).

In 2014, the average PL symptom^{-1} at GS59+14d was 0.27 pg STB %$^{-1}$ which was slightly lower than the 0.37 pg STB %$^{-1}$ at GS59+28d, but not significantly so. There were genotype differences in PL symptom^{-1} at GS59+28d ($p=0.034$), but not at GS59+14d (Fig 7.12b), and when the data sets were combined there were no significant differences between genotypes ($p=0.106$).

The genotype regression slopes between pathogen load and disease symptoms were analysed. In section 7.3.2.1 of this chapter, an ANCOVA analysis determined significant genotype interactions within the pathogen load – disease symptom relationships. There were significant differences between genotypes slopes, but not intercepts in 2012 when genotype regressions were allowed to vary independently. The parent Rialto was used as a reference genotype, and in total there were 22 genotypes with significantly different slopes from Rialto's (Table 7.3). Four of these genotypes had a more planar slope, hence less disease symptom expression per unit pathogen load, and the remaining 18 had steeper slopes (higher symptom expression for each unit pathogen load). There was no genotype interaction or effect within the disease symptom – pathogen load relationship in the 2014 field experiment. Because of this there were no differences in genotype regression slopes (Table 7.4).

Table 7.3. The individual genotype regressions of the pathogen load and disease symptoms relationship in 2012. Significant differences ($p<0.05$) in slope from the reference genotype Rialto (regression $y = 23.7x + 1.30$) are marked as bold. There were no differences in intercepts so p-values are not presented.

Genotype	Intercept % symptoms	Slope PL symptoms^{-1}	P-value (slope)
CxL 14B	0.02	34.1	0.673
CxL 3D	-0.40	39.0	0.086
CxL 41F	1.45	30.1	0.277
CxL 42C	0.73	35.1	0.245
CxL 43A	0.02	44.3	**<.001**
CxL 48D	-0.14	38.0	0.214
CxL 5H	-0.02	25.7	0.840
CxL 6E	-0.01	30.8	0.346
CxL 7a	0.76	42.5	0.253
CxL 8I	-0.31	34.7	0.187
L14 x R 10	3.51	15.0	**0.016**
L14 x R 104	2.89	38.3	**0.002**
L14 x R 107	0.19	38.8	**0.044**
L14 x R 112	1.63	42.9	**0.014**
L14 x R 114	-0.32	82.9	**<.001**
L14 x R 119	-0.50	43.4	**0.012**
L14 x R 121	0.24	29.7	0.830
L14 x R 123	0.32	31.8	0.298
L14 x R 127	-0.63	40.1	**0.035**
L14 x R 129	1.17	34.6	0.140
L14 x R 131	0.58	27.0	0.676
L14 x R 133	0.95	37.2	0.162
L14 x R 136	2.60	26.0	0.712
L14 x R 138	2.92	32.6	0.126
L14 x R 15	0.84	38.1	**0.025**
L14 x R 16	4.43	12.8	**0.006**
L14 x R 19	2.68	15.5	0.075
L14 x R 20	0.54	45.5	**<.001**
L14 x R 22	2.70	12.4	**0.013**
L14 x R 23	1.89	24.7	0.850
L14 x R 35	1.35	18.1	0.416
L14 x R 43	0.51	35.5	**0.031**
L14 x R 5	1.56	37.6	0.056
L14 x R 50	2.02	36.4	**0.027**
L14 x R 55	2.87	16.2	0.133
L14 x R 59	0.10	56.7	**0.001**
L14 x R 62	0.71	45.9	**0.001**
L14 x R 66	0.73	19.6	0.630
L14 x R 68	-0.43	42.7	**<.001**
L14 x R 7	2.15	22.9	0.865
L14 x R 70	2.45	39.9	**0.020**
L14 x R 74	1.61	43.6	**0.004**
L14 x R 80	0.19	45.2	**0.037**
L14 x R 82	1.23	34.8	**0.023**
L14 x R 85	0.70	18.5	0.292
L14 x R 89	0.70	30.9	0.161
L14 x R 91	3.16	15.4	**0.044**
L14 x R 92	0.12	39.5	0.174

Table 7.4. The individual genotype regressions of the pathogen load and disease symptoms relationship in 2014. All assessments were combined into a single analysis. The slope p-values compared to the reference genotype AxC117 (regression y = 15.1x+ 1.34) are shown.

Genotype	Intercept (% Symptoms)	Slope (PL symptom^{-1})	P-value (slope)
AxC 21	0.82	11.9	0.52
CxL 14B	0.27	11.8	0.88
CxL 43A	1.61	8.0	0.16
CxL 5H	0.63	6.9	0.17
CxL 7A	0.81	11.3	0.47
L14xR 114	2.09	26.0	0.39
L14xR 127	2.50	8.2	0.17
L14xR 133	1.71	11.0	0.47
L14xR 16	0.83	9.3	0.22
L14xR 19	3.50	10.2	0.29
L14xR 20	0.54	6.6	0.10
L14xR 22	0.24	16.0	0.84
L14xR 92	1.06	30.9	0.37
L8xR 10	0.25	26.0	0.55
L8xR 86	0.61	11.3	0.59

7.3.4 Does pathogen load explain the variation in yield loss?

In order to determine if pathogen load can explain genotype differences in the disease - yield loss relationship, the variation in yield loss per AUDPC from the population average was calculated for each genotype and compared to the ratio of pathogen load to visual disease symptoms.

The average AUDPC-yield relationship for the populations in 2012 and 2014 was determined by fitting a linear regression to the genotype averages in each year. In 2012, AUDPC (calculated between GS59 and GS59+14d) accounted for 10.3% of variation in yield loss (p=0.014; Fig 7.11a). The predicted mean relationship obtained from the regression analysis predicted that a hypothetical average genotype loses 14.40% yield in the absence of any AUDPC symptoms, followed by a loss of 0.08% grain yield per unit AUDPC.

In 2014, AUDPC (calculated over all three assessments) accounted for 49.1% of variation in yield loss (p=0.001; Fig 7.11b). The predicted mean

indicated that a hypothetical genotype has an initial yield loss of 6.96%, followed by 0.10% yield loss per unit increase in AUDPC.

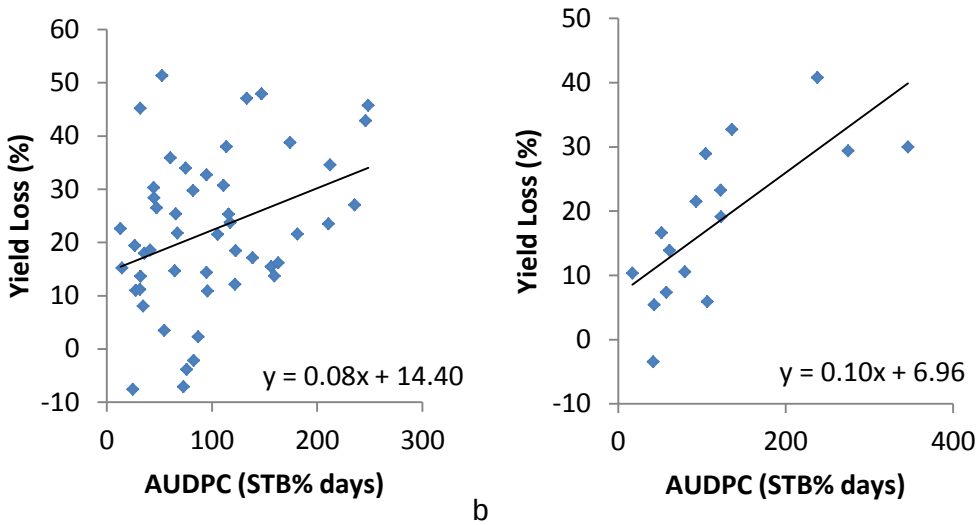


Fig 7.11. The relationship between the genotype averages of percentage yield loss and untreated AUDPC a) AUDPC (calculated between GS59 and GS59+14d) in 2012; and b) in 2014 with AUDPC calculated over three assessments.

The variation from the predicted mean was determined by the y-axis distance of each data point from the regression line. In 2012, the variation ranged from -27% to 33%, i.e. from 27% less yield to 33% more yield lost than predicted. The range in yield loss variation from the predicted mean was smaller in 2014; from -14% to 13%.

In order to determine whether this variation in yield loss could be explained by genotype differences in the non-symptomatic disease to disease symptom ratio, the pathogen load at 5% disease symptoms for each genotype was correlated to the yield loss variation. There was no relationship between the yield loss variation and pathogen load in 2012 or 2014 (Fig 7.12a-b). The pathogen growth rate (i.e. pathogen load increase per unit disease) was also compared to the variation in yield loss, but did not have any impact on yield loss.

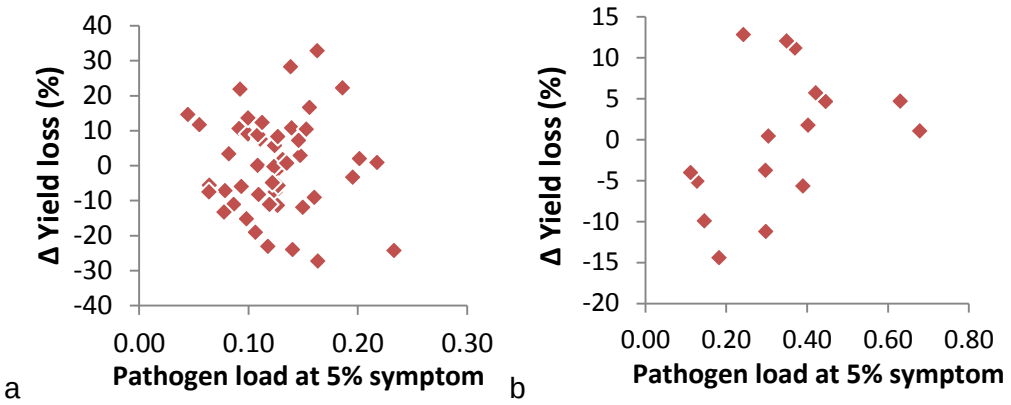


Fig 7.12. The relationships between pathogen load at 5% disease symptoms and genotype variation in yield loss from the predicted mean in a) 2012 and b) 2014.

7.3.5 Does pathogen load explain non-lesion green area loss?

A similar analysis to the AUDPC-yield loss variation (section 7.3.4) was performed in order to determine the influence of in-leaf pathogen load on variation in green area loss– disease symptom relationships.

In 2012, there were significant differences between timings in the GLA loss – STB severity relationship. The overall relationship was strong, with disease severity accounting for 83.0% of variation in GLA loss ($p<0.001$). There was however a significant timing effect, as GS59+14d and GS59+28d had 3.4% and 19.0% higher intercept than GS59. This parallel regression model accounted for 87.7% of variation ($p<0.001$). There was no timing interaction in the relationship when all three assessments were included, but when GS59+28d was excluded there was a near significant timing interaction ($p=0.053$), as GS59+14d had a steeper regression slope than at GS59 (Fig 7.13). Because of this, the timings were analysed separately.

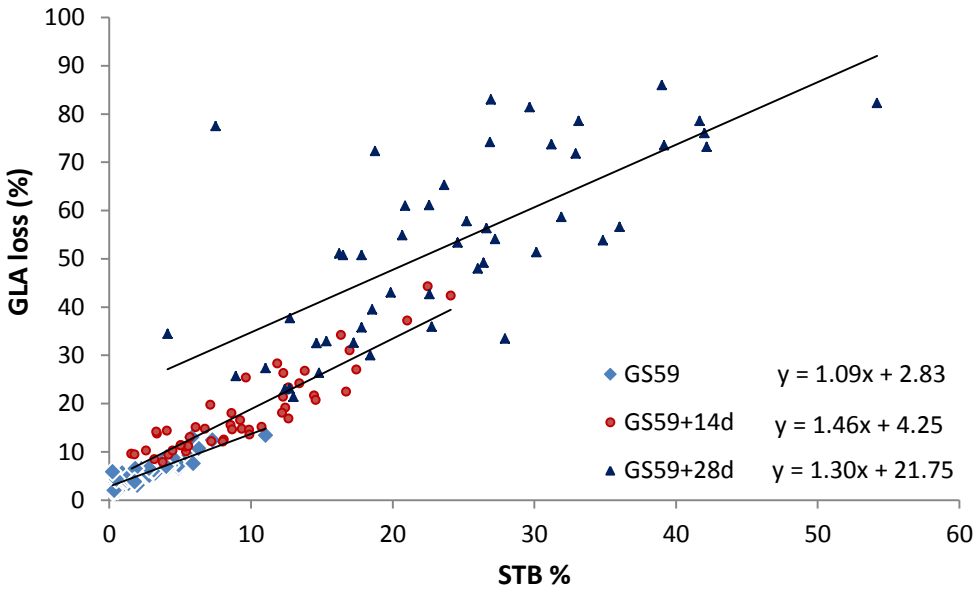
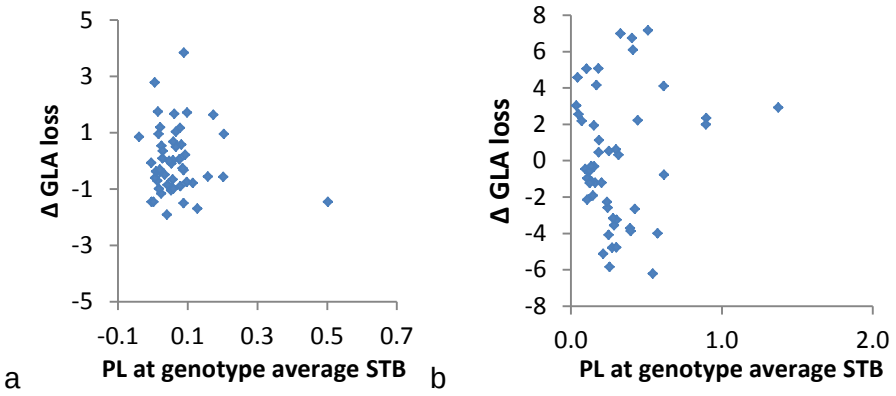


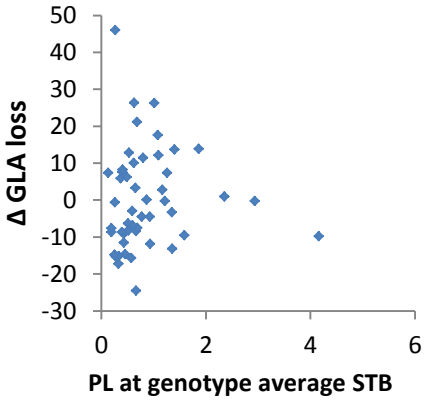
Fig 7.13. The relationship between disease symptom severity and green leaf area loss at GS59 and GS59+14d for in 2012.

There was some variation in the GLA loss compared to the predicted mean at GS59, with genotype averages ranging from 1.9% less GLA loss than predicted to 3.84% more. There was no relationship between genotype pathogen load calculated per 5% STB severity and variation in GLA loss at GS59, or between predicted pathogen load at the actual symptom level and genotype variation in GLA loss (Fig 7.14a).

The genotype range in GLA lost compared to the predicted mean was larger at GS59+14d, ranging from 6.9% less GLA lost than predicted to 7.2% more. There was no relationship between pathogen load per 5% STB severity and variation in GLA loss at GS59+14d, or between predicted pathogen load at the actual symptom level and genotype variation in GLA loss (Fig 7.14b).

At GS59+28d in 2012, the genotype variation in GLA loss from the predicted mean was considerably higher than the previous assessments; from 24.5% less to 46.0% more GLA loss than predicted. As seen in the previous assessments, there was no relationship between genotype variation in GLA loss and pathogen load at 5% symptoms or at the actual symptom level (Fig 7.14c).





c

Fig 7.14. The relationships between predicted pathogen load at the genotype average disease severity and genotype variation in GLA loss from the predicted mean (Δ GLA loss) in a) GS59; b) GS59+14d; c) GS59+28d; in 2012.

In 2014, assessment timing had no effect on the STB and GLA loss relationship, so the two timings (GS59+14d and GS59+28d) were combined into a single analysis. The overall relationship between STB symptoms and GLA loss was quite strong, with STB symptoms accounting for 76.7% of variation ($p<0.001$; Fig 7.15). The genotype GLA losses ranged from 6.7% less than the predicted mean to 13.5% more. There was, however, no relationship between pathogen load at 5% symptoms and genotype variation in GLA loss (Fig 7.16), or between pathogen growth rate (pathogen load increase per unit symptom) and variation in GLA loss.

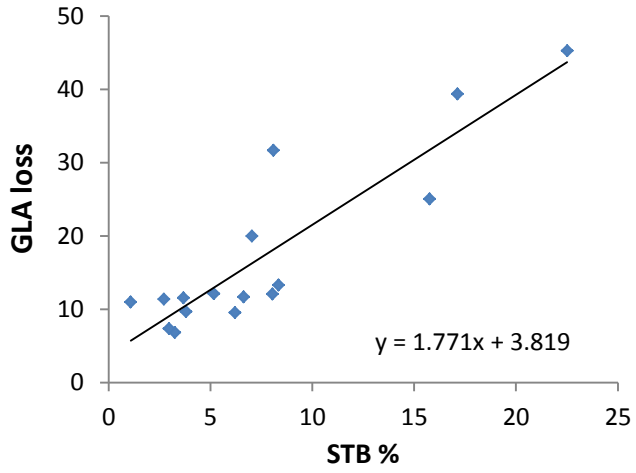


Fig 7.15. The relationship between STB symptom percentage and GLA loss percentage in 2014.

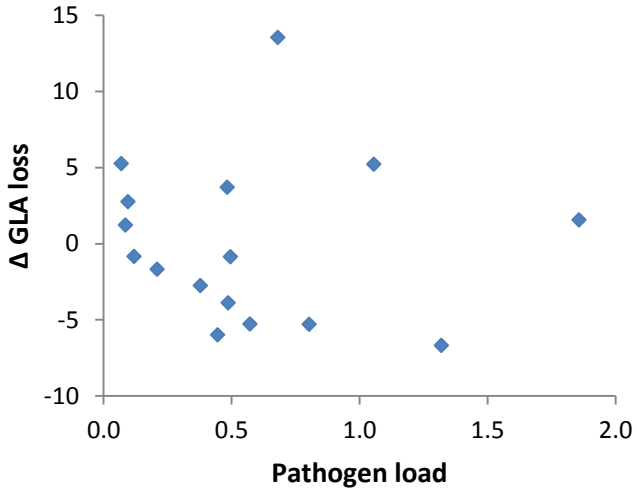


Fig 7.16. The relationship between pathogen load (at the genotype mean % symptom) and genotype variation in GLA loss from the predicted mean (Δ GLA loss) for 16 genotypes in 2014.

7.3.6 Relationship between disease tolerance and pathogen loads

The relationships between the pathogen loads and disease tolerance, as calculated from the HAD in Chapter 4, were determined using linear regression analyses.

In 2012, there was no relationship between disease tolerance and untreated or protected pathogen load at GS59. There was a weak relationship between PL symptoms^{-1} and disease tolerance at GS59 ($p=0.017$, $r^2=0.031$).

This was however due to two genotypes, and when these were excluded from the analysis there was no significant relationship. At GS59+14d, there was no relationship between protected pathogen load and disease tolerance but there was a near-significant (p=0.063), extremely weak negative relationship in the untreated treatment (r^2=0.017). There was no relationship between PL symptoms^{-1} at GS59+14d and tolerance. At GS59+28d, there was a significant but weak negative relationship between untreated pathogen load and disease tolerance (p=0.011, r^2=0.062). In the protected treatment, there was a slightly stronger negative relationship between pathogen load and disease tolerance (p=0.004, r^2=0.116). There was also a weak negative relationship between PL symptoms^{-1} and disease tolerance (p=0.020, r^2=0.051), however when a single genotype was removed this relationship was only near-significant and very weak (p=0.081, r^2=0.024).

In 2012, there was no relationship between disease tolerance and the iPL calculated between GS59 and GS59+14d. There were, however, weak relationships between iPL (GS59+14d and +28d) and disease tolerance (p=0.002, r^2=6.0%), and between iPL calculated over all three assessments and tolerance (p=0.004, r^2=5.1%).

In 2014, there was no relationship between pathogen load and disease tolerance at either treatment or assessment, and there was no relationships between PL symptoms^{-1} and disease tolerance. In addition, there was no relationship between iPL and disease tolerance in 2014.

7.3.7. Calculating disease tolerance using qPCR?

Disease tolerance was calculated using the integrated pathogen load, iPL, by the same method as tolerance would normally be calculated from HAD and AUPDC. The disease tolerance calculated by iPL will be abbreviated tol^{iPL}, and correspondingly the disease tolerances calculated from AUDPC and HAD will be abbreviated tol^{AUDPC} and tol^{HAD} for the remainder of the chapter.

In 2012, as mentioned previously, the entire data set could not be accurately quantified for AUDPC and integrated pathogen load over all three assessments. For this reason, tol^{AUDPC} and tol^{iPL} was first determined for the full data set, using the two first assessments (GS59 and GS59+14d).

When disease tolerance was calculated using iPL, 26 out of the 49 genotypes displayed disease tolerance and 11 displayed intolerance. When calculated with AUDPC; 27 genotypes displayed tolerance and 14 genotypes displayed intolerance. The disease tolerance determined by HAD on the other hand, determined 22 genotypes to be tolerant and 17 to be intolerant.

There were some differences in the ranking of the genotypes, however, particularly between tol^{HAD} and the other two methods. Perhaps most notably, the disease tolerance calculated by HAD determined the UK genotypes (CxL and Rialto) to be either intolerant or neutral, however, disease tolerance calculated by iPL determined all of these to be either tolerant or neutral (Table 7.5).

In 2012, disease tolerance of 23 out of 49 genotypes could be determined for AUDPC and iPL between GS59+14 to GS59 +28d and over all three assessments. In order to compare tol^{iPL} and tol^{AUDPC} to tol^{HAD}, the genotypes were ranked as “tolerant”, “neutral”, and “intolerant” according to the 95% confidence interval for the group of 23 genotypes, as opposed to the entire population. In general, the disease tolerance determined by each method was similar when calculated between the two assessments (GS59+14d; GS59+28d) and over all three assessments. Tol^{AUDPC} determined 10 out of 23 genotypes to be tolerant, as compared to 9 genotypes for tol^{iPL} and 7 genotypes for tol^{HAD} (Table 7.6). Four genotypes displayed disease tolerance calculated by all three methods in 2012 (L14xR20; -43; -68; -80).

In 2014, 14 out of 16 genotypes could be determined for tol^{iPL}. Disease tolerance was also calculated using AUPDC, between GS59+14d and GS59+28d and over all three assessments. As in 2012, the 95% confidence interval was calculated for these 14 genotypes and the genotypes were grouped according to their tolerance level (Table 7.7). In general, tol^{iPL} and tol^{AUDPC} were in more agreement than tolerance based on HAD-values. Each method of calculating disease tolerance identified six genotypes to be tolerant (except tol^{AUDPC} calculated between GS59+14d and +28d) and out of these six genotypes, two displayed tolerance in all three methods (Table 7.7). No genotypes displayed intolerance in all the three methods.

Table 7.5. Comparison of disease tolerance slopes calculated by HAD (t GLA days^{-1}), AUDPC; GS59 to GS59+14d) and int. PL (GS59 to GS59+14d), in 2012. Figures in **bold** indicate genotypes that were significantly tolerant.

Genotype	HAD (t GLAI days^{-1})	Tol	AUDPC (t %STB days^{-1})	Tol	int. PL (t pg STB days^{-1})	Tol
CxL 14B	0.020	N	**-0.154**	T	**-1.384**	T
CxL 3D	0.045	I	-0.130	I	-1.545	N
CxL 41F	0.032	I	**-0.021**	T	**-1.242**	T
CxL 42C	0.022	I	**-0.028**	T	**-0.279**	T
CxL 43A	0.025	I	**-0.056**	T	**-0.858**	T
CxL 48D	0.015	N	-0.043	N	**-0.461**	T
CxL 5H	0.024	I	**-0.042**	T	**-1.256**	T
CxL 6E	0.040	I	**-0.052**	T	**-0.412**	T
CxL 7a	0.038	I	-0.041	N	-0.496	N
CxL 8I	0.019	N	-0.074	I	**-0.502**	T
L14 x R 10	0.026	I	**-0.013**	T	**-0.008**	T
L14 x R 104	0.029	I	**-0.015**	T	**-0.212**	T
L14 x R 107	0.016	N	**-0.018**	T	-0.292	N
L14 x R 112	**0.013**	T	**-0.005**	T	-0.069	I
L14 x R 114	**0.010**	T	**-0.033**	T	0.027	N
L14 x R 119	0.016	N	**-0.043**	T	**-0.289**	T
L14 x R 121	**0.005**	T	-0.015	I	-0.502	I
L14 x R 123	**0.011**	T	-0.056	N	**-0.181**	T
L14 x R 127	**0.012**	T	-0.004	I	**0.040**	T
L14 x R 129	0.024	I	-0.018	I	-0.435	N
L14 x R 131	**0.000**	T	0.000	I	0.012	I
L14 x R 133	0.021	I	**-0.029**	T	-0.368	N
L14 x R 136	0.028	I	**-0.025**	T	-0.118	N
L14 x R 138	**0.010**	T	**-0.007**	T	-0.294	N
L14 x R 15	**0.010**	T	**-0.008**	T	**-0.043**	T
L14 x R 16	0.021	I	**-0.025**	T	**-0.008**	T
L14 x R 19	**0.004**	T	-0.006	I	-0.008	I
L14 x R 20	**0.000**	T	-0.001	N	0.116	I
L14 x R 22	0.050	I	-0.037	I	-0.099	I
L14 x R 23	**0.011**	T	-0.014	I	-0.041	N
L14 x R 35	0.019	N	**-0.016**	T	**-0.055**	T
L14 x R 43	**0.009**	T	-0.006	I	-0.102	I
L14 x R 5	0.020	N	**-0.015**	T	**-0.381**	T
L14 x R 50	0.014	T	**-0.009**	T	**-0.124**	T
L14 x R 55	0.018	N	**-0.021**	T	**-0.039**	T
L14 x R 59	**0.005**	T	-0.006	I	-0.127	N
L14 x R 62	0.016	N	-0.010	N	**-0.098**	T
L14 x R 66	0.017	N	**-0.038**	T	**-0.193**	T
L14 x R 68	**0.004**	T	0.010	I	0.062	I
L14 x R 7	0.021	I	-0.006	N	**-0.005**	T
L14 x R 70	**0.009**	T	-0.011	I	-0.378	I
L14 x R 74	**0.009**	T	**-0.005**	T	**-0.143**	T
L14 x R 80	**0.001**	T	-0.003	N	-0.017	N
L14 x R 82	**0.014**	T	-0.014	N	-0.174	N
L14 x R 85	**-0.001**	T	0.004	I	0.006	I
L14 x R 89	**0.011**	T	**-0.025**	T	-0.323	I
L14 x R 91	0.031	I	**-0.006**	T	**-0.005**	T
L14 x R 92	**0.012**	T	**-0.058**	T	**-0.307**	T
Rialto	0.029	I	**-0.063**	T	**-1.273**	T

Table 7.6. Disease tolerances calculated by HAD (GLAI days), AUDPC (% STB days) and integrated pathogen load (pg STB days) in 2012. The AUDPC and PL tolerance are calculated over both three assessments (GS59; +14d; +28d) and between the last two assessments (GS59+14d and +28d). The genotypes are grouped into tolerant (T), intolerant (I) and neutral (N) according to the 95% confidence intervals. Figures in **bold** indicate genotypes that were significantly tolerant.

	HAD		AUDPC				Int. PL			
Genotype	**All ass.**	**Tol.**	**All ass.**	**Tol.**	**+14 to +28d**	**Tol.**	**All ass.**	**Tol.**	**+14 to +28d**	**Tol.**
CxL 43A	0.025	I	-0.0111	I	-0.0132	I	-0.1195	I	-0.1288	N
L14 x R 104	0.029	I	-0.0077	I	-0.0157	I	-0.0962	N	-0.0906	N
L14 x R 112	0.013	N	**-0.0030**	T	**-0.0067**	T	**-0.0441**	T	-0.0821	N
L14 x R 114	0.011	N	**-0.0030**	T	**-0.0032**	T	-0.0656	N	-0.0924	N
L14 x R 119	0.016	N	-0.0069	N	-0.0081	N	-0.0512	N	-0.0681	N
L14 x R 121	**0.005**	T	-0.0103	I	-0.0234	I	-0.3094	I	-0.7517	I
L14 x R 123	0.011	N	-0.0121	I	-0.0134	I	-0.0662	N	-0.0966	N
L14 x R 127	0.015	N	**-0.0004**	T	**-0.0003**	T	**-0.0117**	T	**-0.0066**	T
L14 x R 129	0.024	I	-0.0093	I	-0.0190	I	-0.1230	I	-0.1748	N
L14 x R 138	0.010	N	-0.0048	N	-0.0120	N	-0.0910	N	-0.1053	N
L14 x R 15	0.010	N	-0.0045	N	-0.0097	N	**-0.0321**	T	**-0.0887**	T
L14 x R 20	**0.000**	T	**-0.0010**	T	**-0.0011**	T	**-0.0066**	T	**-0.0043**	T
L14 x R 43	**0.009**	T	**-0.0031**	T	**-0.0063**	T	**-0.0391**	T	-0.0617	N
L14 x R 5	0.020	I	-0.0080	I	-0.0189	I	-0.1543	I	-0.2359	I
L14 x R 50	0.014	N	-0.0059	N	-0.0153	I	-0.0839	N	-0.1720	N
L14 x R 59	**0.005**	T	**-0.0035**	T	**-0.0072**	T	-0.0641	N	-0.1283	N
L14 x R 68	**0.004**	T	**0.0012**	T	**0.0014**	T	**0.0068**	T	**0.0074**	T
L14 x R 7	0.021	I	**-0.0034**	T	**-0.0047**	T	**-0.0117**	T	**-0.0252**	T
L14 x R 74	**0.009**	T	**-0.0029**	T	**-0.0066**	T	-0.0769	N	-0.0982	N
L14 x R 80	**0.001**	T	**-0.0014**	T	**-0.0024**	T	**-0.0119**	T	**-0.0295**	T
L14 x R 89	0.011	N	-0.0117	I	-0.0218	I	-0.0954	N	-0.1243	N
L14 x R 92	0.012	N	-0.0089	I	-0.0103	N	-0.0902	N	-0.1199	N
Rialto	0.029	I	-0.0128	I	-0.0157	I	**-0.0341**	T	**-0.0341**	T

Table 7.7. Disease tolerances calculated by HAD (GLAI days), AUDPC (% STB days) and integrated pathogen load (pg STB days) in 2014. Tol^{AUDPC} and tol^{iPL} are calculated between the last two assessments (GS59+14d and +28d), and tol^{AUDPC} was also calculated over all three assessments. The genotypes are grouped into tolerant (T), intolerant (I) and neutral (N) according to the 95% confidence intervals. Figures in **bold** indicate genotypes that were significantly tolerant.

	HAD		AUDPC				iPL	
Genotype	**All ass.**	**Tol.**	**All ass.**	**Tol.**	**+14 to +28d**	**Tol.**	**+14 to +28d**	**Tol.**
AxC 117	0.053	I	-0.016	N	-0.024	I	-0.069	N
AxC 21	0.024	N	-0.016	N	-0.019	N	-0.058	N
CxL 14B	**0.024**	**T**	-0.036	I	-0.066	I	-0.235	I
CxL 43A	0.034	I	-0.020	I	-0.024	I	**-0.030**	**T**
CxL 5H	0.028	N	-0.026	I	-0.031	I	-0.111	I
L14xR 114	**0.015**	**T**	-0.015	N	-0.021	N	-0.181	I
L14xR 133	**0.010**	**T**	**-0.004**	**T**	**-0.006**	**T**	**0.012**	**T**
L14xR 16	0.037	I	-0.017	I	-0.019	N	-0.035	N
L14xR 19	0.033	I	**-0.005**	**T**	**-0.007**	**T**	**-0.031**	**T**
L14xR 20	**0.021**	**T**	-0.016	N	-0.021	N	-0.033	N
L14xR 22	0.060	I	**-0.011**	**T**	-0.015	N	**-0.018**	**T**
L14xR 92	0.030	N	**-0.002**	**T**	**-0.004**	**T**	**0.022**	**T**
L8xR 10	**0.000**	**T**	**-0.001**	**T**	**0.000**	**T**	**0.064**	**T**
L8xR 86	**0.023**	**T**	**-0.009**	**T**	**-0.013**	**T**	-0.051	N

7.3.8 Can physiological traits explain disease tolerance calculated from pathogen load?

The final objective was to determine if the method of calculating disease tolerance had an impact on the apparent importance of physiological traits which have been hypothesised to be important in disease tolerance. In order to do this, the disease tolerances of 23 genotypes from 2012 and the 14 genotypes from 2014 quantified by iPL, AUDPC and HAD were re-analysed against selected physiological traits from Chapters 4 and 5 (Table 7.8).

In general, the relationships between disease tolerance quantified by either AUDPC or iPL and physiological traits were quite weak. Tol^{AUDPC} tended to display a higher number of (and stronger) correlations against physiological traits than tol^{iPL}. All methods of calculating disease tolerance in both years indicated that a high fungicide protected yield and grains m^{-2} was related to intolerance (tables 7.8 & 7.9; Fig 7.17a-f). There were also strong relationships between yield loss and all methods of calculating disease tolerance in 2012, but not for tol^{AUDPC} and tol^{iPL} in 2014.

In 2012, tol^{AUDPC} displayed mainly negative relationships with yield components, in particular protected grain yield, but also with grains m^{-2} and grains ear^{-1} (Table 7.8). This method of calculating disease tolerance displayed a positive relationship with protected HAD $grain^{-1}$, but it was still quite weak at 10.9% ($p<0.001$).

Tol^{iPL} in 2012 displayed a few relationships with physiological traits, and these were overall very weak. There were weak negative relationships with yield components such as protected grain yield and grains m^{-2} (Table 7.8). The strongest relationship was between decreased yield loss and increased tolerance (accounting for 22.1%) and the phenotypic trait displaying the strongest relationship with increased tol^{PL} was decreased crop height (10.5%).

In 2014, there were several relationships between source traits and tol^{AUDPC} (Table 7.9); in general, decreased HAD and GLAI in the absence of disease were related to increased tolerance. Decreased canopy size and crop height was also related to tol^{AUDPC}. As expected, tol^{AUDPC} was also related to low fungicide protected grain yield and grains m^{-2} (Table 7.9).

Tol^{iPL} in 2014 displayed fewer and weaker relationships than tol^{AUDPC}. The strongest relationships were found between tol^{iPL} and low fungicide protected

grain yield and grains m^{-2}, but there were also relationships with source traits such as decreased protected GLAI, decreased LAI and crop height and decreased untreated light interception (Table 7.9).

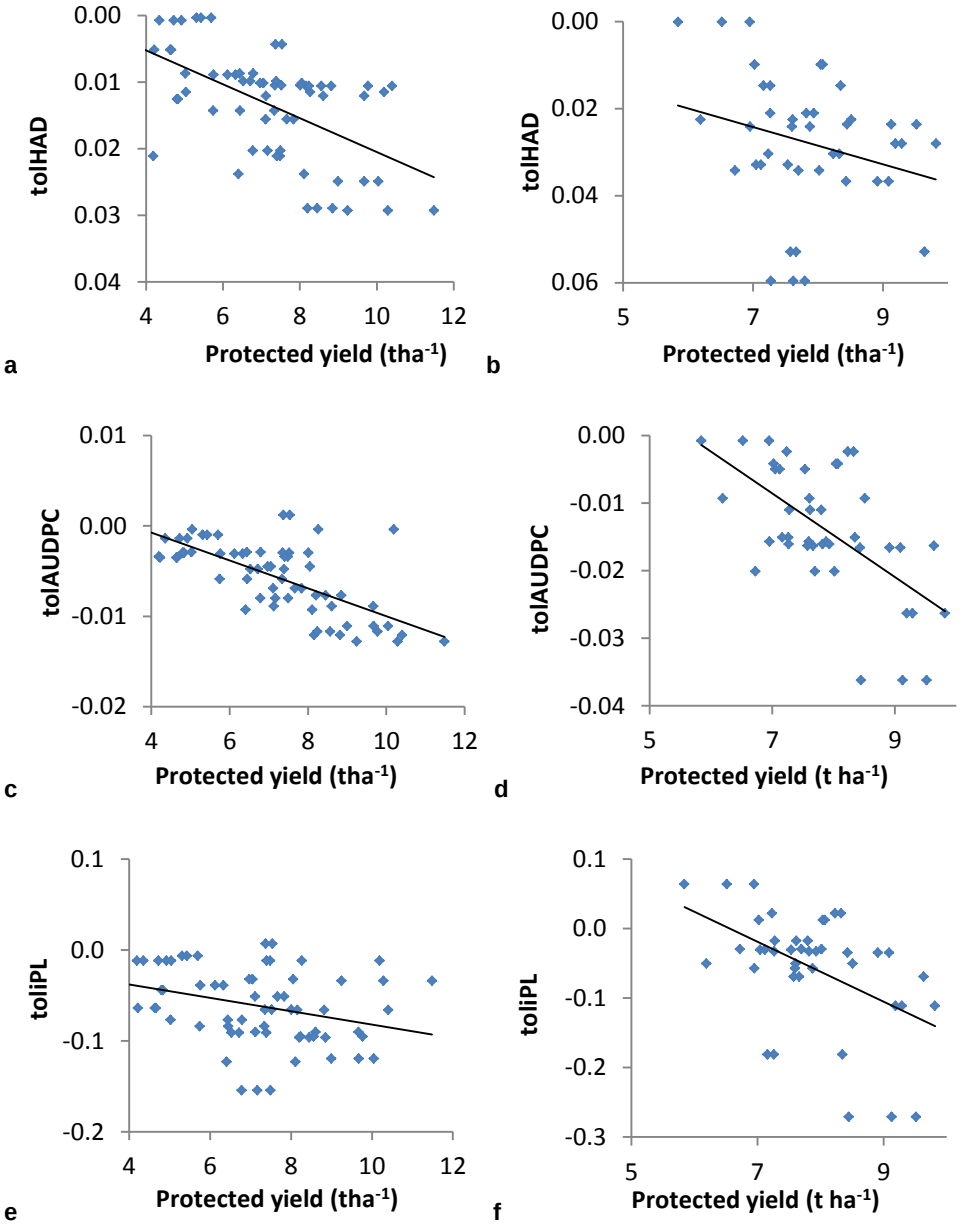


Fig 7.17. The relationships between the disease tolerances and protected grain yield a) tol^{HAD} in 2012; b) tol^{HAD} in 2014; c) tol^{AUDPC} in 2012; d) tol^{AUDPC} in 2014; e) tol^{iPL} in 2012; f) tol^{iPL} in 2014.

Table 7.8. The relationships between disease tolerances calculated by HAD, AUDPC and integrated PL over all three assessments, and selected physiological traits in the 2012 field experiment.

		Tolerance HAD		**Tolerance AUDPC**		**Tolerance iPL**	
Trait	**Fung.**	p	r^2	p	r^2	p	r^2
HAD	U	0.001	13.1% (+)	-	-	-	
HAD	P	-		0.082	3.0% (-)	-	
Grain yield (t ha^{-1})	U	-		0.022	3.9% (-)	-	
Grain yield	P	<0.001	27.3% (-)	<0.001	46.2% (-)	0.010	8.6% (-)
Yield loss (t ha^{-1})		<0.001	44.4% (-)	<0.001	34.3% (-)	<0.001	22.1% (-)
GLAI	U	-		0.039	4.8% (-)	-	
GLAI	P	-		0.005	9.8% (-)	-	
Grains m^{-2}	U	-		0.005	9.9% (-)	-	
Grains m^{-2}	P	<0.001	15.6% (-)	<0.001	22.1% (-)	0.032	5.5% (-)
TGW	U	0.002	11.8% (+)	0.081	3.1% (+)	0.088	3.0% (+)
TGW	P	-		-		-	
Grains ear^{-1}	U	-		0.032	5.3% (-)	-	
Grains ear^{-1}	P	0.004	10.5% (-)	<0.001	11.2% (-)	-	
Canopy size		0.018	3.6% (+)	-	-	0.006	5.2% (+)
LAI		-		0.006	5.0% (-)	-	
Flag-leaf area		0.006	4.8% (+)	0.074	1.6% (+)	-	
Flag-leaf width		-		-		-	
Flag-leaf length		0.007	4.5% (+)	-		-	
Ears m^{-2}		-		-		-	
Had ear^{-1}	U	<0.001	19.0% (+)	-		-	
Had ear^{-1}	P	-		0.073	1.6% (-)	-	
HAD $grain^{-1}$	U	<0.001	18.6% (+)	0.046	4.5% (+)	-	
HAD $grain^{-1}$	P	<0.001	20.7% (+)	0.004	10.9% (+)	0.089	3.0% (+)
Crop height		-		-		<0.001	10.5% (-)

Table 7.9. The relationships between disease tolerances calculated by HAD, AUDPC and iPL and selected physiological traits in the 2014 field experiment.

		Tolerance HAD		**Tolerance AUDPC**		**Tolerance iPL**	
Trait	**FT**	p	r^2	p	r^2	p	r^2
HAD	U	0.005	16.0% (+)	-		-	
HAD	P	0.006	15.4% (+)	<0.001	22.1% (-)	0.098	4.4% (-)
Grain yield	U	0.003	18.1% (+)	0.050	7.0% (-)	0.030	9.0% (-)
Grain yield	P	0.094	4.5% (-)	<0.001	35.5% (-)	0.001	21.4% (-)
Yield loss (%)		<0.001	39.4% (-)	-		-	
GLAI	U	0.017	11.2% (+)	0.032	8.8% (-)	-	
GLAI	P	0.037	8.2% (+)	0.001	20.9% (-)	0.012	12.7% (-)
Grains m^{-2}	U	0.067	5.8% (+)	-		-	
Grains m^{-2}	P	0.022	10.3% (-)	0.001	21.1% (-)	0.002	19.1% (-)
TGW	U	0.004	17.1% (+)	-		-	
TGW	P	-		-		-	
Grains ear^{-1}	U	-		-		-	
Grains ear^{-1}	P	-		-		-	
Canopy size		-		-		-	
LAI		0.051	3.5% (+)	<0.001	15.8% (-)	0.011	6.6% (-)
Flag-leaf area		-		-		-	
Flag-leaf width		-		0.049	3.5% (+)	0.045	3.6% (+)
Flag-leaf length		0.007	7.3% (-)	0.015	5.9% (-)	0.043	3.8% (-)
Ears m^{-2}		-		0.002	9.8%	0.031	4.4% (-)
Had ear^{-1}	U	0.054	6.7% (+)	-		-	
Had ear^{-1}	P	-		-		-	
HAD $grain^{-1}$	U	0.052	6.8% (+)	-		-	
HAD $grain^{-1}$	P	<0.001	27.9% (+)	-		-	
Crop height		-*		<0.001	20.1% (-)*	<0.001	15.1% (-)*
WSC utilisation (g m^{-2})		<0.001	17.9% (-)	-		-	
Light interception (GS59)	U	<0.001	22.1% (+)	0.029	9.2% (-)	0.032	8.7% (-)
Light interception (GS59)	P	0.087	4.8% (+)	-		-	
Light interception (GS71)	U	<0.001	34.7% (+)	-		-	
Light interception (GS71)	P	0.011	13.1% (+)	-		-	
RUE (pre-anthesis)		-		0.087	2.4% (+)	-	
RUE (post-anthesis)	U	-		-		-	
RUE (post-anthesis)	P	0.097	4.4% (+)	-		-	
Biomass (anthesis)		-		-		-	
Biomass (PM)		0.002	9.6% (+)	0.088	2.3% (-)	-	
Harvest index	U	-		-		-	
Harvest index	P	0.006	15.2% (-)	-		-	

* L8xR10 excluded because of extreme height (134 cm)

7.4 Discussion

In this study, an attempt was made to determine the impact of pathogen load on disease-yield relationships. As discussed in Chapter 6, few studies have targeted the visual symptom vs. in-leaf pathogen biomass relationship for STB in field experiments. Fraaije et al. (2001) found a relationship between the average number of pycnidia on flag leaves and average STB DNA in field experiments using a non-sequence specific SYBR green qPCR assay. Gouache et al. (2011) found relationships between thermal time from symptom onset and pathogen load. The only field-based study directly comparing necrotic leaf area caused by STB was Rohel et al. (2002). These authors, however, reported that there were at times considerable differences between visual symptoms and detected pathogen load in the field experiments. One reason for the relative lack of in-field disease studies quantifying the pathogen load vs. disease symptom relationship could be the background noise introduced in field experiments, and the difficulty in interpreting the results that accompany this.

The first hypothesis of this chapter was that there would be differences in pathogen load between genotypes. This was true as there were significant differences in the actual pathogen load between genotypes at all assessments even at ear emergence in 2012. The genotype differences were, however, due to a single, extremely susceptible genotype (L14xR10) at GS59 and GS59+14d. This genotype displayed nearly ten times higher pathogen load than the average at ear emergence; 0.61 pg compared to the average 0.08 pg, and also higher than average disease symptoms at this assessment (11% compared to the average 3%; Appendix Table A4.d). However, it is possibly attributable to the background noise in the 2012 dataset that there were no other genotype differences identified, as the genotype range was quite large even when L14xR10 was excluded. The 2014 dataset was less variable and there were more reliable pathogen load differences, as the outcome of the statistical analysis was not influenced by a single genotype.

It is possible that additional variability was introduced to the 2012 samples due to the sampling method. In this year the leaves were sampled by three 0.5 cm^2 cores evenly distributed along the leaf. It is likely that the cores would not be representative at very low disease presence, as the cores could miss any lesions present on the leaves. This is likely to be one of the reasons why

the pathogen load disease-symptoms were much stronger in the final assessment when there was more disease present as discussed below.

One of the main objectives of this chapter was to determine the general relationship between in-leaf pathogen load and visual disease symptoms in field samples. In general, there was little agreement between visual disease symptoms and pathogen load in the early assessments, and particularly so at ear emergence in 2012. This was to some extent expected as a low amount of pathogen target DNA is more difficult to quantify accurately using qPCR (Ellison et al., 2006), and could also be due to the sampling method as mentioned above. At the final assessment in 2012 the relationships were quite strong, probably as a combination of a higher amount of disease at the final assessments being easier to quantify confidently by the use of qPCR, and a higher density of pathogen load within the leaves ensuring that the sampling is more likely to encounter disease lesions.

The disease symptom – pathogen load relationship was generally stronger in the 2014 field experiment, which is likely to be due to the sampling method. In order to compare the assessments on the sampled leaves to in-field assessments on random tillers, both types of assessments took place. The assessments on the sampled leaves correlated much more strongly with the pathogen load which was expected; however, the in-field assessment still accounted for approximately the same amount of variation as in 2012.

The hemibiotrophic nature of *Z. tritici* means that there could be non-symptomatic disease present which could affect the pathogen load – visual symptom relationships. Due to this, it was expected that the overall pathogen loads in the populations would be consistently higher than corresponding disease symptoms (and fall to the right in the regression graphs, see illustration in Fig 7.18). This was not the case in 2012, in which the pathogen load to disease symptom ration tended to be low and cluster to the left of the graphs, particularly in the first two assessments. In 2014, the disease symptom – pathogen load ratios tended to be slightly more balanced.

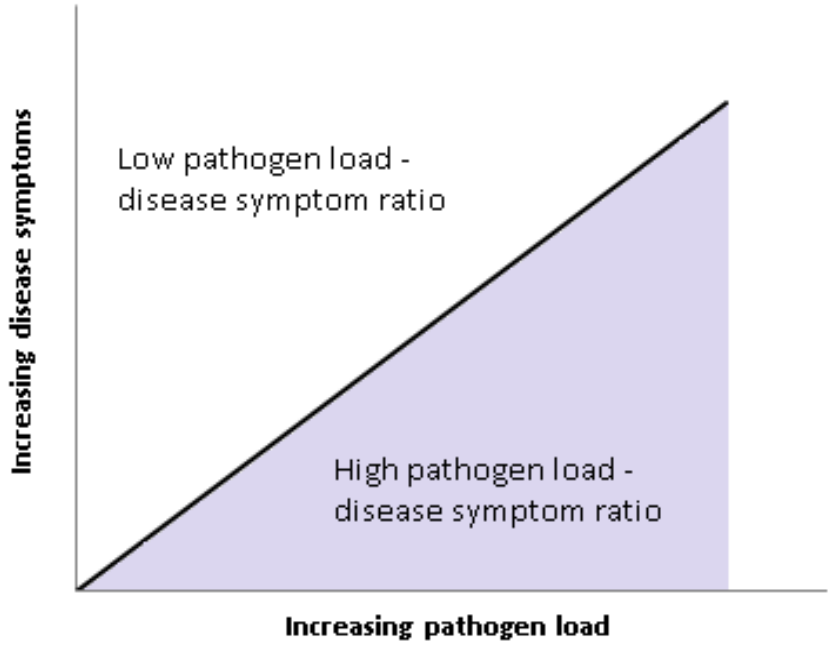


Fig 7.18. Illustration of high/low pathogen load to disease symptom ratio and where these fall in a graph compared to a perfectly balanced ratio.

The second hypothesis of this chapter stated that there are genotype differences in the disease symptom – pathogen load relationship. This certainly seems to be the case as there were highly significant covariate (genotype) interactions in at all assessments in 2012, and for the lab-based assessment in 2014. A genotype interaction indicates that there are significant differences within a population in both intercepts and slopes of the genotype regression between pathogen load and disease symptoms. Essentially this means that the genotypes must be analysed individually, and no general disease prediction can be obtained from the population. While this interaction complicates the development of predictions systems, it is much more interesting on a plant pathology – crop physiology level and has not been extensively investigated previously.

The parent Rialto was used as the reference genotype in all 2012 assessments and had a linear regression function symptom area = 23.7PL + 1.3 which means that at no detectable pathogen load there is an initial 1.3% disease symptoms, which then increases 23.7% per unit increase in pathogen load. Two examples of genotypes for which pathogen load – disease symptom relationships was significantly different from Rialto are L14 x R16 ($p=0.001$) and L14 x R20 ($p=0.004$; Table 7.3). According to the regression slopes, L14 x R16 displays less visual symptom increase for each unit

pathogen load than Rialto, and also less visual symptoms at any pathogen load above approximately 0.3 pg compared to Rialto (Fig. 7.19). On the other hand, L14xR20 displayed high visual symptoms at low pathogen load compared to Rialto, and has a considerably higher increase in visual symptoms for each increasing unit pathogen load. L14 x R20 was determined as tolerant and L14 x R16 as intolerant in both field seasons (based on HAD-values, see Chapter 4). There was no overall relationship between the genotype disease symptom – pathogen load regression slopes and disease tolerance, but all four genotypes displaying a significantly lower slope value than Rialto (Table 7.4) displayed intolerance in the 2012 experiment, and 15 out of 18 genotypes displaying significantly higher slopes were determined as tolerant.

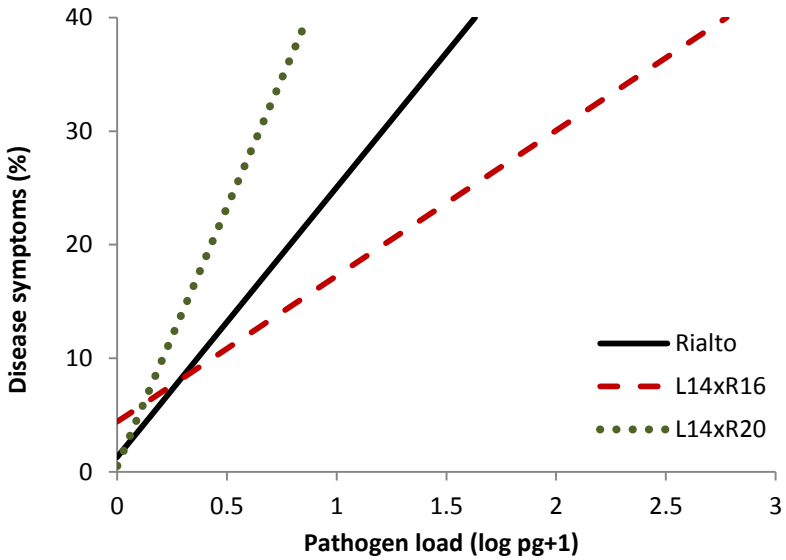


Fig 7.19. Three examples of genotypes contrasting in pathogen load –disease symptom relationships.

The third hypothesis stated that high pathogen load to visual disease symptom ratio will cause increased yield loss and increased development of non-lesion green area loss. As discussed above, the pathogen load – disease symptom relationship is highly variable and in order to choose a representative genotype value to use in the analyses, the pathogen load per 5% disease symptom was calculated for each genotype according to the individual regression slopes. The pathogen load per unit symptom did not seem to explain the variability in the disease – yield relationship, despite the large genotype variation in yield loss. In fact, in 2012 a genotype that lost approximately 30% more yield than the predicted average had the same

amount of pathogen load as a genotype that lost about 30% less yield than expected (Fig 7.13a). This also seems to be the case for non-lesion green area loss; despite large variation in green area loss per unit disease symptoms (particularly at the later assessments) there did not seem to be a relationship with increased pathogen load. There is almost certainly an impact of disease but it might not be as straightforward as a quantitative relationship of increased pathogen biomass and increased green area loss. Based on these results it is more likely to be induced by metabolic costs of pathogen presence and/or restrictions on a single leaf basis. It has been shown that leaf necrosis caused by STB can increase leaf respiration, which could potentially be due to stimulation of defence mechanisms (Robert et al., 2006) On a crop level, potential metabolic or defence costs are likely to impact grain yield, as is restricted green area.

The fourth hypothesis states that there will be a relationship between pathogen load per unit symptoms and disease tolerance. As could be expected based on the lack of relationship between pathogen load and yield loss and green area loss, this was not the case. As there has been very little research performed on the pathogen biomass – yield loss relationships, it could be hypothesised that the apparent disease tolerance is an outcome of a partial resistance mechanism – in which symptoms were allowed to appear but the in-leaf growth was restricted to various degrees. However, except for weak relationships at the final assessment in 2012, in which high in-leaf pathogen load was related to intolerance (accounting for 6% and 11% in the untreated and protected treatment respectively), there was little evidence present to confirm this.

Finally, the last hypothesis stated that there will be differences when disease tolerance is calculated by HAD, AUDPC and pathogen load, and that these disease tolerances will be related to different physiological traits. The main differences were found between disease tolerance calculated by HAD and disease tolerances calculated by AUDPC and integrated pathogen load (iPL). This was not surprising as firstly; there were fairly strong relationships between integrated pathogen load and AUDPC, and secondly; both of these quantify the actual disease presence as opposed to green area loss. In general, it was hard to draw conclusions about the disease tolerance levels of genotypes. There were no genotypes that displayed consistent tolerance/intolerance between methods and site-seasons (see Tables 7.5, 7.6

&7.7), although some did show consistent tolerance levels over methods within a single year

The main physiological traits analysed in Chapter 5 were re-analysed against disease tolerance calculated by HAD (as previously) and also by AUDPC and iPL. In agreement with the hypothesis, there did seem to be differences between which traits displayed relationships with the different methods of calculating disease tolerance. In particular, disease tolerance calculated by HAD contrasted strongly against disease tolerance calculated by AUDPC and iPL. However, there was one trait that all methods (in both years) determined as a disease tolerance conferring trait: low grain yield in the absence of disease – essentially low yield potential. This in turn, seems to be conferred by a low number of grains m^{-2} and, at least in 2012, low number of grains ear^{-1}. This was not surprising as low yield potential has been discussed as the main drawback of disease tolerance (Bingham et al., 2009) and seem to be consistently identified in disease tolerance studies (Parker et al., 2004, Paveley et al., 2005, Foulkes et al., 2006).

The biggest differences between disease tolerance calculated by HAD vs. disease tolerance calculated by AUDPC and iPL were the relationships with source traits. The disease tolerance calculated by HAD had nearly exclusively positive (or neutral) relationships with source traits, in 2012 primarily with untreated HAD and in 2014 with HAD and GLAI in both treatments, and strong relationships with high light interception. There were also positive relationships with source-sink traits, such as high untreated HAD ear^{-1} and HAD $grain^{-1}$. Overall, disease tolerance calculated by HAD could be generalised as: low yield potential, high source capacity. In contrast, the disease tolerances calculated by AUDPC and iPL had general negative relationships with source traits; for instance with GLAI, LAI, fungicide protected HAD and untreated light interception (at ear emergence). A generalisation of disease tolerance calculated by AUDPC and iPL could be low yield potential and low source capacity.

Nearly all current hypotheses about which traits confer disease tolerance are based on source capacity and resource interception and use. For instance, high source-sink capacity conferred by HAD per grain was suggested by (Paveley et al., 2008), and large flag leaves, canopy size and kPAR has also been suggested (Foulkes et al., 2006, Bingham et al., 2009,

Parker et al., 2004). These hypotheses stem from observations in disease tolerance studies based on HAD calculations, which is essentially a measure of source capacity over time. It is not entirely unlikely that some of the relationships between HAD-based disease tolerance and source traits are affected by the method of disease tolerance calculation.

7.5 Conclusion

In this study, an attempt was made to determine the impact of pathogen load on disease-yield relationships. There were genotype differences in the pathogen load – visual disease symptoms relationship, in which both the amount of visual symptoms development per unit pathogen load and the increase of visual disease symptoms per unit pathogen load increase differed significantly. Although this is not surprising, it has not been shown previously and could potentially improve the precision of disease predictions. Genotype differences in pathogen load – disease symptom relationships did not seem to be related to the disease - yield loss relationship directly or cause increased non-lesion green leaf area loss. This was in contrast to the hypotheses that increased pathogen load would increase yield loss per unit disease symptoms and non-lesion green leaf area loss due to increased metabolic costs.

Differences in the genotype tolerance/intolerance ranking could be seen when disease tolerance were calculated based on different measures of disease symptoms; HAD, AUDPC and pathogen load integrated over time. However, the disease tolerance calculated by AUDPC and integrated pathogen load was reasonably similar, but contrasted strongly with disease tolerance calculated by HAD. Few genotypes were determined to be consistently tolerant/intolerant by all three methods of calculating disease tolerance, and no genotypes were consistent over both methods and site-seasons. There were also differences in which traits conferred disease tolerance when HAD-based tolerance was compared to AUDPC and iPL-based tolerance. HAD-based tolerance indicated that a large source capacity conferred by trait such as large HAD, GLAI and light interception are beneficial for maintaining yield in the presence of disease. However, AUDPC and iPL-based tolerance indicate that a small source capacity is related to high disease tolerance. All methods did however confirm previous findings of disease tolerance being related to low yield potential.

8. General Discussion

8.1 How is disease tolerance conferred?

Disease tolerance, the ability to maintain grain yield in the presence of disease (Parker et al., 2004), was investigated in this thesis. It has been proposed that disease tolerance could confer durable and broad-spectrum defence against pathogens, while exerting little or no selection pressure on pathogen populations (Bingham et al., 2009). Due to this, selection for disease tolerance in breeding programmes could be useful but before this can be implemented, selectable physiological traits for disease tolerance screening need to be identified.

Disease tolerance has been studied for many years, despite the low numbers of peer-reviewed publications available. Some of the early research indicated that traits such as up-regulated photosynthesis in healthy leaf tissues could buffer for green area losses caused by disease, and potentially confer disease tolerance (Williams and Ayres, 1981, Scholes and Farrar, 1986). These types of traits could be candidate tolerance traits but were not within the scope of this thesis. This section of the discussion will focus on in-field adult plant traits exclusively. There are few field-based experiments published which investigate physiological traits potentially conferring disease tolerance in wheat (Ziv and Eyal, 1976, Ziv et al., 1981, Parker et al., 2004, Foulkes et al., 2006, Bancal et al., 2015). In early studies conducted in Israel, disease tolerance was mainly quantified as low percentage yield loss when diseased (Ziv and Eyal, 1976). The later in-field studies quantified disease tolerance as the slope between grain yield DM and HAD in units of t ha^{-1} GLAI day^{-1}, the method used in this thesis.

In this study, a large range of disease tolerance was identified in 2012. The range in disease tolerance slopes found in this study ($-0.1*10^{-2}$ to $5.0*10^{-2}$ t ha^{-1} HAD^{-1}) was larger than the corresponding slopes previously reported by Parker et al. (2004), which ranged between $1.4*10^{-2}$ to $2.3*10^{-2}$ t ha^{-1} HAD^{-1}. The large variation in disease tolerance was mainly due to the genotype population investigated; a DH mapping population derived from a cross between the Mexican spring wheat Line 14 and the UK winter wheat Rialto. The parental Line 14 was selected for high yield in the CIMMYT shuttle breeding program, which incorporates alternative cycles of selection in

disease presence which could confer disease tolerance. This particular population was selected for disease tolerance studies because of contrasting sink and source traits. Line 14 is a large-ear phenotype and because of this has a high sink capacity while Rialto has shown high resource-use efficiency and water soluble stem carbohydrate accumulation, and therefore a large source capacity (Shearman et al., 2005, Gaju et al., 2014). The L14 x Rialto DH lines and two DH lines of a CIMMYT Line 8 x Rialto DH population (all of which referred to as CIMMYT lines) displayed a wide range of disease tolerance. In both field experiments, all significantly tolerant genotypes had CIMMYT backgrounds while there were both CIMMYT and UK backgrounds in the intolerant genotypes.

It has been well documented that there is a potential association between low grain yield potential and disease tolerance (Bingham et al., 2009), and this has been found in previous experimental studies (Parker et al., 2004, Foulkes et al., 2006). In this study, there was a relationship between a low protected yield and disease tolerance in both years, although the association was weak in 2014. Bancal et al. (2015) also found contrasting relationships between fungicide-protected yield and disease tolerance between site-seasons, as there was no relationship in two of the experiments but one experiment displayed a reasonably strong association with decreased grain yield. Grain yield can generally be divided into grains m^{-2} and thousand grain weight (TGW) and there tends to be a negative relationship between these two components (Slafer et al., 1996). Present field experiments indicated that there was a negative association between protected grains m^{-2} and disease tolerance. Grain number m^{-2} was strongly related to protected grain yield, and therefore likely to be involved in the negative association between protected grain yield and disease tolerance. Lower grains m^{-2} could be associated with a greater extent of sink limitation of grain yield which would favour disease tolerance. In accordance with the grains m^{-2} and TGW balance, field experiments indicated a positive association between TGW and disease tolerance. The relationships between disease tolerance and grains m^{-2} and TGW agree with most of the literature. For instance, Ziv et al. (1981) suggested that increased TGW could be a selectable trait for disease tolerance and Parker et al. (2004) found that high grain numbers m^{-2} was associated with low disease tolerance. However, a recent study found that

TGW was associated with intolerance and suggested that breeding efforts should be focused on increased grains ear^{-1} (Bancal et al., 2015).

A major physiological component of yield formation is harvest index, the ratio of grain weight to total above-ground dry matter. In this study, disease tolerance was associated with both low harvest index and high total AGDM in the absence of disease. As would be expected from the low harvest index, a growth analysis at physiological maturity indicated that a high combined stem and leaf weight was associated with disease tolerance, both as actual weight and as proportion of total dry matter. There is little literature available to compare the AGDM-disease tolerance relationship. Paveley et al. (2008) mentions finding a non-significant but positive relationship between AGDM accumulation and disease tolerance, while this was not presented or studied by Foulkes et al. (2006) and Bancal et al. (2015).

The CIMMYT population investigated in this study had lower average protected yield than the UK-adapted genotypes, and also lower grains m^{-2}. This was not surprising as these genotypes are adapted to a climate that is very different from the growth conditions in NW Europe. Wheat grown in high radiation, irrigated conditions tend to be sink limited which could be determined by grains m^{-2} (Reynolds et al., 2005), while wheat grown in the dull, humid conditions of NW Europe may have a tighter balance between source and sink capacity (Beed et al., 2007). It has previously been suggested that a high source to sink balance could be beneficial for disease tolerance (Bingham et al., 2009), and it is therefore possible that the apparent associations in this study between low grains m^{-2} and disease tolerance and maintained TGW and disease tolerance are effects of sink limitation of grain growth as mentioned above.

Apart from the actual yield components, there was a range of physiological traits associated with disease tolerance although several of these were quite weak. The strongest relationship in this study was for protected HAD grain^{-1} in 2014, which accounted for 33.6% of the variation in disease tolerance. However, many of the relationships accounted for around 5-10% of variation. The relatively weak associations between disease tolerance and physiological traits agree with some of the literature, for instance Bancal et al. (2015) analysed a large range of physiological traits and found correlations ranging between 16 and 43% of variation accounted for.

The exception is the study performed by Foulkes et al. (2006), in which high correlations between disease tolerance and selected physiological traits (grain yield, grains m^{-2} and stem WSC) were observed, accounting for between 43 and 73% of variation. However, it is not likely that a single trait accounting for nearly all of the variation will be identified. This is because disease tolerance is likely to be conferred by a combination of physiological traits (and underlying QTLs), and there is no distinct line between tolerance and intolerance.

In general, the field experiments indicated that increased source capacity, particularly conferred through HAD and GLAI in the presence of disease, was conducive to disease tolerance. This corresponds with hypotheses outlined in this thesis and in several publications (Parker et al., 2004, Paveley et al., 2008, Bingham et al., 2009, Ney et al., 2013), and associations between increased HAD and disease tolerance were also found in all field experiments reported by Bancal et al. (2015). In this study, there was a weak positive association between untreated LAI at ear emergence and disease tolerance in the 2014 field season and hence between tolerance and larger canopy size. Bancal et al. (2015) found considerably stronger relationships between increased LAI and disease tolerance in their study.

An association between radiation interception and disease tolerance has been widely hypothesised (Parker et al., 2004, Bingham et al., 2009, Ney et al., 2013) either through increased canopy size or by a high light extinction coefficient (k_{PAR}). In this study, there was no relationship between disease tolerance and k_{PAR} and this was also the case for the study conducted by Foulkes et al. (2006). There was, however, a relationship between fractional light interception and disease tolerance, particularly at GS73 in the presence of disease. The increased fractional light interception was mainly conferred through a larger canopy size, consistent with the lack of association between k_{PAR} and disease tolerance.

It is clear that source capacity has a large impact on disease tolerance and a high source to sink ratio has been suggested as a potential tolerance mechanism (Paveley et al., 2008). HAD $grain^{-1}$ is a measure of source:sink ratio that seems to display consistently positive associations with disease tolerance. In this study, protected HAD $grain^{-1}$ accounted for 20% and 34% of the genetic variation in disease tolerance in 2012 and 2014, respectively. This

is close to values found in the literature, for instance Paveley et al. (2008) and Bancal et al. (2015) reported HAD grain^{-1} accounting for 40% and 39% of variation in disease tolerance, respectively. It is reasonable that a high amount of source to sink capacity is beneficial to disease tolerance; a crop with a high amount of excess source capacity can afford to lose a larger fraction of green area to disease than a crop with a tightly balanced source:sink capacity.

The final major physiological trait to discuss is the amount of stem water soluble carbohydrates (WSC). Increased WSC was suggested as a candidate disease tolerance trait by Parker et al. (2004) as it could potentially buffer the effect of post-anthesis green area losses, and this was tested in field experiments by Foulkes et al. (2006). These authors did, however, find that there was a strong negative correlation between WSC stem storage at anthesis and disease tolerance (Foulkes et al., 2006). In this study there was a weak negative association between WSC stem storage at GS59 + 7d and disease tolerance. However, there was a positive association between remaining amount of WSC at physiological maturity and disease tolerance, and in consequence, an association between low WSC utilisation and disease tolerance. In this study, there was no association between accumulated WSC at physiological maturity and grain yield, TGW or grains m^{-2}, with the exception of a weak association between untreated grain yield and WSC. This indicates that the association between high WSC at physiological maturity and disease tolerance is not due to autocorrelation of both of these traits with grain yield.

The association between disease tolerance and WSC stem storage at physiological maturity has not previously been shown. This association was stronger in the fungicide-protected treatment and could possibly be an effect of an increased source capacity in combination with low sink capacity. The increased WSC storage at physiological maturity was associated with both an increased HAD and a prolonged period between ear emergence and canopy senescence. Potentially, an increased green canopy size and prolonged grain-filling period could lead to an excess of photosynthates that are not translocated to the grain, if the crop is limited by low grain numbers. The CIMMYT genotypes had higher average WSC stem storage at both anthesis and physiological maturity, and displayed increased HAD and HAD per grain^{-1}

in 2012. These sink-limited genotypes might produce a larger excess of assimilates due to greater sink limitation.

To summarise, it is clear that a high source capacity and low sink capacity is a major influence of the disease tolerance in this study, and can also explain the association between a limited yield potential and disease tolerance. However, in 2012 six CIMMYT genotypes displayed both significant disease tolerance and higher than average yield. One of these genotypes (L14xR123) yielded over 9 t ha^{-1} in the protected treatment, which was also above the average grain yields of the UK genotypes. This genotype displayed a large HAD, above average grains m^{-2} and TGW, and in addition; high HAD $grain^{-1}$. Unfortunately, this genotype was not included in the 2014 field experiments, but future disease tolerance studies should be performed on genotypes displaying these traits.

In order for disease tolerance to be introduced to breeding programmes the potential yield limitation needs to be circumvented. To identify potential disease tolerance traits that are not effects of sink limitation, studies should focus on genotypes displaying reasonably high protected yield and grains m^{-2}, and more balanced source:sink ratios. As there was generally variation in the associations between traits and disease tolerance between years, heritable traits with stable disease tolerance associations need to be identified. Based on both this study and literature, HAD $grain^{-1}$ seems to display the most stable relationship with disease tolerance, and is not necessary linked to a low yield potential as demonstrated by the abovementioned genotype L14xR123.

8.1.1 Which factors influence the slope between healthy area duration and grain yield?

As discussed throughout this dissertation, there is no universal method of quantifying disease tolerance. Current measures of disease tolerance tend to quantify disease presence using absolute values of canopy green area over time (as HAD) and yield loss (as t ha^{-1}) (Parker et al., 2004, Bingham et al., 2009).

The use of HAD as a predictor of grain yield was initially proposed by Waggoner and Berger (1987), who suggested that quantifying light interception and absorption throughout the season was sufficient for yield

predictions. A decade later, Bryson et al. (1997), showed that the HAD: yield relationship differ between winter wheat varieties – and therefore essentially differing in disease tolerance. Although the HAD: grain yield model is arguably the best current disease tolerance model, using HAD calculations have certain disadvantages. For instance, there is an inability to distinguish between crops that has a large GLAI but early senescence and crops with small GLAI and late senescence (Bryson et al., 1997). Senescence, particularly when caused by STB, tends to begin in the lower canopy and continue up the canopy – with the flag leaf as the last remaining leaf. As the upper two leaves, and particularly the flag leaf, are the primary determinants of grain yield (Paveley et al., 2012); a crop with prolonged flag leaf duration, even though it has a low GLAI, would have an advantage over a crop with high initial GLAI but short flag leaf duration.

In addition, HAD calculations do not account for the light interception of separate leaf layers. The importance of separate leaf layers differs according to canopy architecture; a crop with prostrate flag leaf will intercept the majority of light in the flag leaf layer, while crops with erect leaves will allow light to disperse further down the canopy. Detailed canopy architecture traits such as flag leaf angles are not feasible to incorporate for large screening experiments. However, as HAD is calculated as the integral of the GLAIs of each leaf layer over time (Bryson et al., 1997); it should be possible to incorporate weighted importance to each leaf layer in the calculations, assigning the flag leaf layer highest importance and decreasing the importance for each subsequent leaf layer.

In regard to quantifying grain yield for disease tolerance, the use of actual yield loss as t ha^{-1} (as opposed to percentage yield loss) may cause low yielding genotypes to appear more tolerant as the actual grain yield lost per percentage yield loss will be smaller. This could be illustrated by the following scenario. Two hypothetical genotypes (A and B) both lose 50% of grain yield and 50% of HAD to disease (Fig 8.1a). Genotype A yields 10 t ha^{-1} and genotype B yields 4 t ha^{-1} in the absence of disease, which means that their actual grain yield loss would be 5 and 2 t ha^{-1}, respectively. Because of this, genotype A will appear more intolerant than genotype B. To further illustrate this, a third hypothetical genotype (C) that also yield 10 t ha^{-1} in the absence of disease could be introduced (Fig 8.1b). This genotype loses 20%

of grain yield to disease, i.e. 2 t ha^{-1}, but will appear equally tolerant to genotype B despite only losing a fraction of the yield.

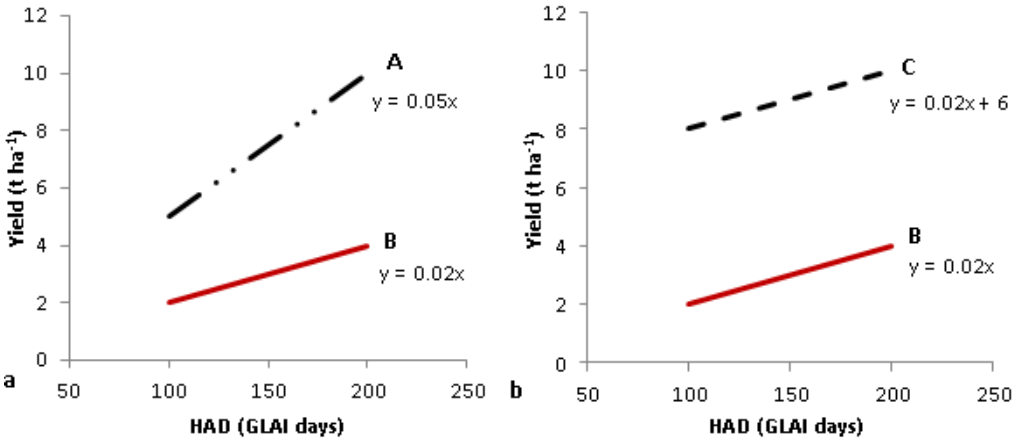


Fig 8.1. Illustration of the appearance of disease tolerance in hypothetical genotypes. Disease tolerance slopes are shown for each genotype. A) Two genotypes losing 50% grain yield; B) Two genotypes losing 2 t ha^{-1} in grain yield.

Future disease tolerance studies will need to account for both actual loss of grain yield and percentage loss of grain yield in order to differentiate between low yield loss due to low yield potential and low yield loss due to disease tolerance. This is could be particularly important when analysing traits conferring disease tolerance to reduce the influence of varieties which seem tolerant due to low yield potential but are not truly tolerant. In order to remove these types of varieties, and index which takes into account both high tolerance and high yield potential should be developed. By removing the effects of low yield potential, the apparent association between low yield potential and disease tolerance (which could be due to inadvertently selecting for low yielding varieties instead of truly tolerant varieties) could potentially be reduced.

8.2 Variation in the L14 x Rialto population

The main reason for choosing the L14 x Rialto DH population was the contrast in source and sink traits within the population. Source-sink traits and balance are, as discussed repeatedly throughout this thesis, candidate disease tolerance traits. The parent Rialto has been shown to have high radiation use efficiency (Shearman et al., 2005) and the parent L14 was

developed by CIMMYT as a large-spike phenotype. The focus of this dissertation has mainly been on identifying physiological traits displaying clear phenotypes that could be incorporated into future breeding programs. However, there is a large genetic variation within this population that could be studied further in regards to affecting disease tolerance.

Firstly, the large-spike phenotype of L14 is conferred in part by a tiller inhibition (*tin*) gene and in part by the introgression of exotic genetic material such as *Agropyron elongatum* (Gaju et al., 2014). Rialto lacks the *tin* gene and although the full L14 x Rialto DH population segregates for this gene, only one of the 39 lines included in the study (L14 x R112) contains the *tin* gene. This line displayed significant disease tolerance, but no conclusions can be made about the relationship between the *tin* gene and disease tolerance without expanding the experiment further.

The L14 x Rialto population also segregates for the dwarfing gene, *Rht-B1*. Both parents contain the dwarfing allele *Rht-D1b*, but L14 also carries *Rht-B1b* (short phenotype) while Rialto contains *Rht-B1a* (tall phenotype). As would be expected from the segregation of dwarfing alleles in the population, there was a large variation in crop height; ranging from approximately 63 cm to 105 cm in 2012. Flintham et al. (1997) found that the presence of *Rht-D1b* reduced the height of near-isogenic lines (NILs) segregating for dwarfing genes to about 83% of the tall controls, while the combination of both *Rht-D1b* and *Rht-B1b* yielded crops of about 56% height. In this study, the presence of the *Rht-B1b* allele reduced the average crop height from 88 cm to 74 cm in 2012. In addition, the presence of *Rht-B1b* seemed to be beneficial to disease tolerance; a line carrying *Rht-B1b* lost on average 0.016 tonne grain yield per unit HAD lost, while a cultivar with *Rht-B1a* lost 0.022 tonne grain yield. This is in contrast to previous disease tolerance studies on *Rht-D1* carried out by Paveley et al. (2005) and Foulkes et al. (2006) in which no effect on disease tolerance was found. It should be noted, however, that the direct relationship between disease tolerance and crop height was quite weak; decreased crop height accounted for only 7% of the variation in disease tolerance.

Finally, the L14 x Rialto population also segregates for photoperiod sensitivity. As L14 is a spring wheat, it carries the alleles *Ppd-D1a* (photoperiod insensitivity) and Rialto carries *Ppd-D1b* (photoperiod sensitivity). The subset of genotypes included in this study were selected for

late flowering and a majority of these are therefore likely to carry *Ppd-D1b*, but there were some variation in development between the genotypes. In 2012, ear emergence varied between 31st of May and 13th of June. The genotypes that were grouped into the "early development" group displayed increased tolerance compared to the genotypes of the "late development" group as the early genotypes lost on average 0.018 tonne grain yield per unit HAD and the late genotypes lost an average of 0.024 tonnes.

It is possible that the increased disease tolerance in early-flowering genotypes is due to all of these belonging to the L14xRialto population and a majority of the late genotypes having UK-adapted backgrounds. On the other hand, the early genotypes had an average of 6 days longer canopy duration from ear emergence to canopy senescence in 2012 compared to late and middle groups of genotypes. It is generally believed that increased canopy duration, particularly increased flag-leaf green area duration, increases grain weight and consequently grain yield. Although there were no convincing relationships between increased canopy duration and any yield component, there were strong relationships with untreated HAD, HAD ear^{-1} and HAD $grain^{-1}$ in both years. In addition, there was a relationship between increased canopy duration remaining stem WSC at pre-harvest in 2012. All of these traits are associated with increased disease tolerance and accordingly the canopy duration period accounted for 30% of variation in disease tolerance in 2014, but only for 5% of variation in 2012.

It is likely that early development and increased grain-filling duration is beneficial for disease tolerance. By allowing an extended period of canopy photosynthesis it is likely to increase the accumulation of source assimilates, which would explain the relationship with excess stem WSC at pre-harvest. Again, this corresponds with the hypothesis that disease tolerance is, at least to some extent, conferred by sink limitation and excess source capacity.

8.3 Is in-leaf pathogen load of importance?

Ney et al. (2013) introduced the concept of "integrated fungal mass" compared to symptom area as a first step in predicting disease tolerance. Their hypothesis was that a tolerant genotype would display low symptom-pathogen biomass ratio (Ney et al., 2013) which is essentially a high pathogen load per unit symptom area. Although not explicitly stated, this hypothesis is

likely to stem from decreased restrictions of green area loss, due to decreased necrosis formation. Conversely, it has been shown that leaf pathogens generally affect photosynthesis in a larger area than the actual lesion size. This ratio of the “virtual lesion” to visual lesion was quantified by Bastiaans (1991), who found that the rice blast fungus virtual lesion was 3-4 times the size of the visual lesion as measured by the area of reduced photosynthesis. Robert et al. (2006) found that the size of the virtual lesion for STB depended on the developmental stage of the lesion itself, and that the virtual lesion was 1.35 times the size of the visual lesion post-necrosis development. The work of Roberts et al. (2006) was, however, conducted on a single wheat cultivar so it is not clear whether the size of the virtual lesion associated with STB varies between cultivars. If the virtual lesion size does vary with cultivars, it may explain some of the differences seen in symptom area:yield loss or HAD:yield loss relationships.

In this study there were clear differences in the relationship between in-leaf pathogen load and visual disease symptoms between genotypes in both controlled environment and field experiments. This indicates that there are differences in the response to pathogen infection in different wheat cultivars. All symptoms in the controlled environment experiments appeared at similar times in the various genotypes within a single inoculum concentration, so differences in pathogen growth rates are not likely to be conferred by differences in the latent period, which is defined as the period from spore deposition to symptom appearance (Armour et al., 2004). It is possible that these differences are due to genetic variation in partial disease resistance. As the genotype L14xR127 displayed seedling disease resistance in the controlled environment experiments and disease tolerance in the 2012 field experiment, it is possible that the two disease defence mechanisms are not entirely separate - at least at different stages in the life cycle of the crop. It is generally accepted that there will be a trade-off in yield potential caused by disease resistance, although the actual yield penalty can vary depending on the number of genes and their defence mechanisms (Brown and Rant, 2013). Assuming that partial resistance has some role in the varying degrees of in-leaf pathogen restriction, there could potentially be small, additional fitness costs adding to the apparent trade-off between yield loss and disease tolerance.

Despite the clear genotypic variation in in-leaf pathogen load and visual disease symptoms no relationship could be found between pathogen load per unit symptom and green area loss or between pathogen load per unit symptom and yield loss due to disease. This may be a result of the more tolerant genotypes in this study potentially being sink-limited and therefore relatively insensitive to loss of photosynthetic capacity post anthesis. The relationship between pathogen load per unit symptom and yield loss due to disease should be studied further in populations displaying variation in tolerance where source and sink are in closer balance or grain growth is source limited.

In this study in-leaf pathogen biomass was quantified, however, the location of the increased pathogen biomass was not identified. The location of the pathogen biomass, i.e. within the lesion area, within chlorotic/necrotic leaf or within green leaf was not identified nor was the virtual lesion size in terms of the impact on photosynthetic activity of the leaf. It would be useful to measure these factors in future studies to identify potential metabolic costs to the plant and/or photosynthetic costs that could impact grain yield.

8.4 Comparison of methods of calculating disease tolerance

There are few and relatively weak associations between physiological traits and disease tolerance in the literature, as discussed above. For this reason, disease tolerance quantified by alternative disease severity measures was analysed against physiological traits in order to determine whether novel associations could be determined. Neither of the two disease measures used for this, AUDPC and integrated pathogen load (iPL), in in-field disease tolerance studies in wheat have previously been reported in the literature.

There were large differences between the disease tolerance calculated by HAD and the tolerances determined by AUDPC and iPL. The main differences between these tolerances are illustrated in Fig 8.2, but as a general statement low yield potential and low source capacity appear to confer disease tolerance based on AUDPC and iPL, while low yield potential but high source capacity were most associated with disease tolerance based on HAD measurements. As the HAD-based disease tolerance was discussed in detail previously in this chapter, the remainder of the section will discuss disease tolerance calculated from AUDPC and iPL.

Both AUDPC and integrated pathogen load tolerance were negatively related to source-related traits (GLAI, light interception etc.) and grain number per m^2 which would generally be considered a sink trait. This indicates an overriding effect of low yield potential on tolerance, the negative relationship with crop height might also be indicative of lower HI for the more tolerant genotypes. In addition, there was no evidence of decreased disease presence (AUDPC or iPL) being associated with high or low disease tolerance.

It is clear that the method of calculating disease tolerance has a large impact on experimental outcomes, perhaps more so than previously suggested (Bingham et al., 2009). The fact that all methods indicate that a low grain yield in the absence of disease is associated with disease tolerance strengthens the need to restrict the search for tolerance traits to genotypes with high yield potential in the environment in which they are being grown.

8.5 Brief summary of hypotheses

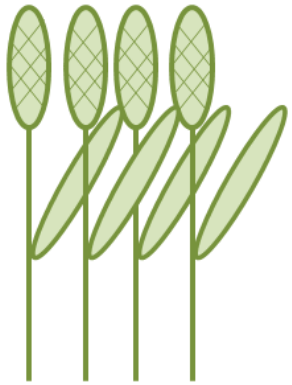
In this thesis, a number of specific hypotheses have been investigated. These hypotheses and brief conclusions for each of these are listed below.

1. The doubled-haploid lines derived from crosses between Rialto and CIMMYT spring wheat advanced lines of large ear phenotype have increased disease tolerance compared to UK adapted material.

   It was shown that a number of the genotypes with CIMMYT backgrounds had increased disease tolerance compared to the UK-adapted genotypes in both field experiments, as all significantly tolerant genotypes had CIMMYT backgrounds.

2. Disease tolerance in wheat is heritable and will be consistently expressed in genotypes over sites and seasons.

   This was only partially true. Out of the four tolerant genotypes in 2014, three had previously displayed disease tolerance and one had been classified as intolerant.



Crop level



Plant level

Disease tolerance
HAD

↑ Light interception (untr.)
↑ GLAI and LAI (untr.)
↓ Grains m^{-2} (prot.)
↓ Grain yield (prot., 2012)
↑ HAD $grain^{-1}$

Disease tolerance
AUDPC

↓ Light interception at GS59 (untr.)
↓ GLAI
↓ Grains m^{-2} (prot.)
↓ Grain yield (prot.)
↑ HAD $grain^{-1}$ (2012)
↑ TGW (2012)
↓ HAD (prot.)

Disease tolerance
Pathogen load

↓ Light interception at GS59 (untr.)
↓ GLAI and LAI (2014)
↓ Grains m^{-2} (prot.)
↓ Grain yield (prot.)

↑ Stem-leaf partitioning at physiological maturity
↑ Stem WSC at physiological maturity
↓ Ear partitioning at physiological maturity
↓ Harvest index (prot.)
↓ WSC utilisation
↓ Grains ear^{-1}

↓ Crop height

↓ Crop height

Fig 8.2. A Summary of physiological traits associated with disease tolerance calculated by the contrasting methods.

3. It is possible to identify variation in disease tolerance amongst genotypes independent of grain yield potential.

   There was an association between low fungicide-protected grain yield and disease tolerance in 2012, but also a number of genotypes displayed both high disease tolerance and high fungicide-protected grain yield. Although there were no genotypes displaying both high disease tolerance and high grain yield in 2014, there was only a weak association between fungicide-protected grain yield and disease tolerance in this year.

4. An increase of green canopy area at ear emergence will be beneficial for disease tolerance as for a given level of disease severity there will be a larger amount of photosynthetic area remaining post-infection to maintain radiation interception.

   There were associations between large green canopy area as conferred by GLAI at ear emergence and disease tolerance amongst genotypes in 2014. There was also an association between a high fractional light interception at ear emergence and disease tolerance in the untreated fungicide treatment.

5. A higher light extinction coefficient will be beneficial to disease tolerance, as it would increase light interception in the top two leaves relative to lower leaves, due to the typically greater disease severity in the lower canopy having a lesser opportunity to impact on radiation interception and yield.

   There was no evidence of an association between the light extinction coefficient and disease tolerance amongst genotypes in this study.

6. A high source to sink balance as indicated by post-anthesis healthy area duration (HAD) per grain in uninfected crops is beneficial for disease tolerance.

   This hypothesis seemed to be true as there were positive associations between HAD grain^{-1} and disease tolerance in both years and in both fungicide treatments. However, genetic variation in HAD grain^{-1} was partly due to differences in sink limitation and yield potential of these genotypes in the environments in which they were tested.

7. A high capacity for water soluble carbohydrate (WSC) stem storage is beneficial for disease tolerance as a buffer for loss of photosynthetic capacity due to disease-induced green area loss during grain filling.

   There was a weak negative association between WSC stem accumulation at GS59+7d, but a positive association between remaining WSC at physiological maturity and disease tolerance. The positive association between WSC at physiological maturity and tolerance is potential another indication of sink limitation in tolerant genotypes.

8. There will be a relationship between visible disease symptoms and in-leaf pathogen load for a given genotype (in controlled environment experiments).

   There were in general relationships between disease symptoms and pathogen loads, but not all of these were linear. These relationships were stronger later in the disease epidemic as pathogen load increased in the latent period when the disease was asymptomatic.

9. The ratio between symptom expression and pathogen load will differ between wheat genotypes, resulting in apparent tolerance or intolerance based on visible symptoms.

   There were significant differences between genotypes when disease symptoms were scored on the same leaves as analysed for pathogen load, and the genotypes varied in both disease expression per unit quantified pathogen load, and the disease symptom increase for each unit pathogen load. This indicates that there may be differential effects of asymptomatic pathogen load between genotypes, which could be causing apparent tolerance or intolerance when it is calculated based on visual disease symptoms. If these effects are mediated through increased non-lesion green area loss then calculating tolerance based on green area remaining will overcome these effects. However, if asymptomatic pathogen load is exerting and increased metabolic cost on the plant or decreasing net photosynthesis in apparently healthy green tissue then the tolerance calculations based on visual assessment of pathogen or green area will be able to account for these effects. Whilst in this study there was no evidence for a direct association between an increased pathogen load per unit symptom and non-lesion green area loss or intolerance such effects may have

been masked by the overriding effect of yield potential on tolerance in the genotypes tested, and this would be worthy of further testing in genotypes with a narrower range of yield potential.

10. Disease tolerance calculated by pathogen load will differ from disease tolerance calculated by AUDPC and HAD, and there will be differences in in which physiological traits affect disease tolerance calculated from pathogen loads, as compared to HAD measurements.

    There were considerable differences between disease tolerance calculated by HAD compared to disease tolerance calculated by AUDPC and integrated pathogen load (iPL). There were fewer differences between disease tolerance calculated by AUDPC and iPL. There were few genotypes that were consistently determined as tolerant/intolerant over all three methods of calculating disease tolerance. In addition, there were differences in the physiological traits associated with disease tolerance according to the different methods of disease tolerance. In general, disease tolerance calculated by AUDPC and iPL was associated with decreased source capacity, while HAD-based disease tolerance was related to increased source capacity. All of the methods were, however, related to low yield potential.

8.6 Directions for future studies

To date, a number of disease tolerance studies have attempted to identify traits conferring disease tolerance. Ideally, these traits should be heritable and be associated with high yield potential in order to be used in breeding programmes. This objective has only been somewhat successful, and this includes the research performed in this study where the overriding effect of yield potential on tolerance may have masked other useful traits. In order to determine the underlying mechanisms conferring disease tolerance, an inter-disciplinary approach might be needed. A potential step by step approach to achieving this could be:

- Screening of mapping populations, preferably using populations that are mapped for genetic linkage, over several site-seasons in order to identify consistent displays of high/low disease tolerance.
- Genotypes displaying consistent high/low disease tolerance should undergo in-depth phenotyping focusing on a small number of selected

traits, again over several site-seasons. Based on this study, these traits could be GLAI at anthesis, post-anthesis light interception and biomass formation (including detailed partitioning), and canopy structure at anthesis. High-throughput field screening methods for these traits including application of canopy sensors, e.g. spectral radiometers for measurements spectral reflectance indices indicative of green canopy area, should also be developed.

- Genotypes investigated in field experiments could also be studied in controlled-environment experiments to complement genetic variation in field-based traits. Some potential objectives could be:
  - Determining the level of disease resistance in genotypes and whether this is associated with in-field disease tolerance. In addition, differential gene expression studies of genotypes displaying high and low disease tolerance during pathogen infection could provide insight on disease tolerance.
  - Quantifying the relationship between pathogen load and symptom development (as in this study) after which a selection of genotypes displaying high/low pathogen loads per unit symptoms could be incorporated into in-field phenotyping for disease tolerance.
  - The combination of pathogen load quantification and determination of metabolic costs of pathogen presence and/or restriction, for instance by measuring changes in photosynthetic capacity due to pathogen load. This could be determined on a whole leaf scale and also in sections of increasing distance from pathogen lesions to determine potential non-symptomatic pathogen spread and the effect on photosynthesis.
- Genetic linkage mapping could be undertaken to determine potential QTLs affecting disease tolerance and associated traits.

9. Appendices

Appendix Table A4.a. Summary of plant developmental dates and crop heights in 2013.

Genotype	Ear emergence (GS59)	Untreated physiological maturity	Protected physiological maturity	Crop height
Avalon	20130617	28 Jul	28 Jul	79.8
Badger	20130624	21 Jul	21 Jul	85.3
Battalion	20130624	21 Jul	21 Jul	76.9
Buster	20130624	21 Jul	21 Jul	75.7
Charger	20130624	21 Jul	21 Jul	76.3
Claire	20130624	21 Jul	21 Jul	79.7
Consort	20130624	21 Jul	21 Jul	77.7
L8xR 10	20130617	28 Jul	28 Jul	115.7
L8xR 11	20130617	28 Jul	28 Jul	95.4
L8xR 14	20130617	28 Jul	28 Jul	69.5
L8xR 16	20130617	28 Jul	28 Jul	74.9
L8xR 20	20130617	28 Jul	28 Jul	68.9
L8xR 28	20130617	28 Jul	28 Jul	81.7
L8xR 34	20130617	28 Jul	28 Jul	78.5
L8xR 43	20130617	28 Jul	28 Jul	91.6
L8xR 44	20130617	28 Jul	14 - 28 Jul	74.4
L8xR 49	20130617	28 Jul	28 Jul	63.0
L8xR 51	20130617	28 Jul	28 Jul	82.4
L8xR 54	20130617	28 Jul	28 Jul	88.8
L8xR 57	20130617	28 Jul	28 Jul	64.5
L8xR 60	20130617	28 Jul	28 Jul	78.5
L8xR 64	20130617	28 Jul	28 Jul	84.6
L8xR 67	20130617	28 Jul	28 Jul	88.2
L8xR 71	20130617	28 Jul	28 Jul	84.8
L8xR 74	20130617	28 Jul	28 Jul	91.7
L8xR 80	20130617	28 Jul	14 - 28 Jul	90.9
L8xR 84	20130617	28 Jul	28 Jul	91.8
CxL 48D	20130624	21 Jul	21 Jul	87.4
CxL 14B	20130624	21 Jul	21 Jul	95.3
CxL 3D	20130624	21 Jul	21 Jul	96.1
CxL 42C	20130624	21 Jul	21 Jul	72.6
CxL 5H	20130624	21 Jul	21 Jul	63.8
CxL 7A	20130624	21 Jul	21 Jul	78.3
Malacca	20130624	21 Jul	21 Jul	80.2
Moulin	20130624	21 Jul	21 Jul	83.4
Oakley	20130624	21 Jul	21 Jul	76.4
Option	20130624	21 Jul	21 Jul	76.7
Rialto	20130624	21 Jul	21 Jul	82.7
Savannah	20130624	21 Jul	21 Jul	81.7
Xi19	20130624	21 Jul	21 Jul	85.5

Appendix Table A4.b Septoria tritici blotch severity in 2013. Symptoms of Septoria tritici blotch throughout the grain filling period of field season 2013. Significance limit is p<0.05 and shown in bold font in table, but near-significant values (p<0.10) are included using normal font.

Year	Observation	Leaf Layer	Average (%) Untreated	Average (%) Protected	SQRT Disease untreated (%)	SQRT Disease protected (%)	P treatments SED (df)	P genotypes SED (df)	P G*E interactions SED (df)
2013	GS59	1	0.03	0.00	0.062	0.011	NS *	NS *	NS *
		2	0.14	0.00	0.21	0.008	**0.016** 0.0256 (2)	**<0.001** 0.100 (156)	**<0.001** 0.141 (156)
		3	0.99	0.01	0.775	0.011	**0.010** 0.078 (2)	**<0.001** 0.205 (156)	**<0.001** 0.290 (156)
	GS59+14	1	0.30	0.00	0.340	0.008	**0.045** 0.0732 (2)	**<0.001** 0.150(156)	**0.001** 0.217 (156)
		2	1.60	0.01	1.04	0.032	**0.020** 0.146 (2)	**<0.001** 0.202 (156)	**<0.001** 0.285 (156)
		3	8.25	0.05	2.55	0.08	**0.013** 0.290 (2)	**<0.001** 0.298 (156)	**<0.001** 0.421 (156)
	GS59+28	1	1.86	0.01	1.11	0.011	**0.021** 0.162 (2)	**<0.001** 0.263 (154)	**<0.001** 0.373 (154)
		2	6.70	0.00	2.32	0.006	**0.004** 0.139 (2)	**<0.001** 0.375(143)	**<0.001** 0.530 (143)

Appendix Table A4.c. Septoria tritici blotch disease incidence on each leaf layer at each assessment during the seasons 2011/2012, 2012/2013 and 2013/2014.

			Disease incidence (%)	
Year	**Assessment**	**Leaf layer**	**Untreated**	**Protected**
2012	GS59	1	97.3	60.1
		2	100	73.5
		3	100	97.3
	GS59 + 14 days	1	100	75.5
		2	100	82.7
	GS59 +28 days	1	100	84.4
2013	GS59	1	15.0	3.3
		2	38.3	2.5
		3	75.8	2.5
	GS59 + 14 days	1	48.3	2.5
		2	68.3	0.8
		3	94.2	14.2
	GS59 +28 days	1	84.9	2.5
		2	93.7	0.9
2014	GS59	1	20.8	37.5
		2	85.4	62.5
		3	100	97.9
	GS59 + 14 days	1	100	79.2
		2	100	93.8
		3	100	100
	GS59 +28 days	1	100	75.0
		2	100	100
		3	100	100

Appendix Table A4.d. Genotype average for STB visual disease symptoms (%) on each leaf layer in the 2012 field experiment. Shown are disease scores in the untreated and fungicide-protected treatment at each disease assessment.

	GS59						GS59+14d				GS59+28d	
	L1		**L2**		**L3**		**L1**		**L2**		**L1**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**
L14xR10	11.0	0.5	16.6	2.5	19.9	10.1	24.1	1.4	34.2	5.3	7.5	4.0
L14xR104	3.7	0.7	15.0	3.8	21.3	7.5	22.5	1.3	19.6	3.5	26.9	2.5
L14xR107	4.0	0.2	8.8	0.9	16.1	1.8	6.1	0.4	13.2	1.4	31.2	3.6
L14xR112	4.5	1.3	13.4	5.0	20.8	6.8	14.6	0.4	25.5	2.1	12.4	2.9
L14xR114	0.9	0.1	4.6	0.7	11.1	1.1	3.4	0.2	8.7	0.4	22.8	1.7
L14xR119	1.4	0.1	5.8	0.5	15.7	1.3	5.4	0.1	17.4	0.7	20.7	0.6
L14xR121	0.3	0.1	1.6	0.4	17.4	8.8	3.3	0.4	14.7	0.2	4.1	0.3
L14xR123	1.2	1.5	3.6	1.6	9.0	2.6	2.6	0.6	12.2	0.7	14.8	1.3
L14xR127	0.3	0.3	2.7	0.1	9.6	2.8	3.2	0.0	13.4	0.9	15.3	0.1
L14xR129	1.8	0.2	7.7	0.1	16.3	4.3	9.9	0.4	20.9	2.3	12.7	0.6
L14xR131	3.2	0.3	8.9	0.3	18.2	4.6	7.2	0.1	22.9	0.3	14.6	1.0
L14xR133	3.1	0.6	8.5	1.6	18.2	7.2	8.6	0.2	20.0	1.2	25.2	2.8
L14xR136	5.9	0.3	16.2	0.4	21.0	1.5	16.7	0.1	28.8	0.4	39.0	3.2
L14xR138	5.8	0.5	17.0	4.6	28.2	8.8	16.4	1.7	41.0	4.7	16.2	1.4
L14xR15	2.2	0.1	10.7	0.8	20.2	5.2	12.4	0.1	28.3	0.6	17.2	0.3
L14xR16	3.9	0.4	14.5	1.3	23.3	7.8	12.3	0.3	24.5	2.9	42.0	5.2
L14xR19	5.1	0.4	10.8	0.8	21.3	3.1	12.3	0.5	23.9	1.7	39.2	2.1
L14xR20	3.0	0.1	6.7	0.0	13.5	1.6	5.4	0.3	15.0	2.2	27.2	1.6
L14xR22	5.2	0.5	13.6	0.4	17.8	4.0	13.8	0.9	25.6	1.8	54.2	1.4
L14xR23	3.2	0.1	11.4	0.4	18.4	2.6	11.9	0.3	21.4	1.3	32.9	3.0
L14xR35	2.8	0.0	7.1	0.1	17.5	0.8	6.8	0.1	16.3	0.0	30.1	1.2
L14xR43	2.0	0.1	6.1	0.1	17.5	3.2	14.5	0.2	32.2	1.0	18.5	0.5
L14xR5	4.9	0.1	15.2	1.5	21.5	3.2	13.4	0.5	18.1	1.8	17.8	1.4
L14xR50	4.9	0.7	12.8	4.9	16.9	8.3	17.4	0.9	26.7	5.1	16.5	1.0
L14xR55	4.3	0.0	12.0	0.6	21.2	5.1	12.2	0.5	26.4	1.0	33.1	1.7
L14xR59	0.8	0.0	4.3	1.0	10.8	8.6	9.2	0.4	20.7	0.9	11.0	0.8
L14xR62	3.8	0.5	18.0	6.7	33.6	6.1	21.0	0.5	26.5	7.8	12.8	1.4
L14xR66	2.3	0.3	8.5	0.3	14.2	1.0	4.1	0.4	16.7	2.0	24.6	0.9
L14xR68	0.7	0.0	4.9	0.0	11.9	0.6	4.2	0.1	12.2	0.3	27.9	1.2
L14xR7	3.9	0.4	13.5	2.5	16.7	8.4	9.7	0.5	21.1	1.6	19.9	4.1
L14xR70	6.2	0.2	14.8	0.1	19.7	0.8	12.7	0.4	28.8	0.5	23.6	1.0
L14xR74	4.1	0.1	8.9	0.7	15.0	5.9	12.7	0.5	26.6	3.2	17.8	0.4
L14xR80	0.5	0.5	5.7	2.8	13.9	4.7	8.6	0.3	18.9	1.7	8.9	0.2
L14xR82	7.3	0.2	14.6	0.4	17.8	0.2	10.8	0.1	19.3	0.8	41.7	1.4
L14xR85	2.8	0.0	9.2	0.2	17.5	0.8	8.0	0.2	20.7	0.5	20.9	0.9
L14xR89	0.6	0.0	5.1	0.4	16.0	2.0	9.4	1.7	19.4	4.7	18.4	2.8
L14xR91	6.3	0.6	10.1	1.0	19.9	11.3	17.0	0.8	33.3	4.1	18.8	3.3
L14xR92	0.5	0.4	2.8	0.4	8.3	2.0	1.6	0.2	9.6	0.1	13.0	0.7
CxL14B	0.2	0.0	4.2	0.2	12.7	0.6	1.8	0.1	7.1	0.4	22.6	0.8
CxL3D	0.4	0.0	7.4	0.1	15.4	1.5	4.5	0.2	14.3	0.2	36.0	1.0
CxL41F	4.0	0.2	12.2	0.1	21.7	1.7	8.7	0.5	24.0	0.9	42.2	1.4
CxL42C	2.8	0.2	10.9	0.4	17.0	4.4	7.1	0.4	19.5	1.2	22.6	2.1
CxL43A	1.3	0.0	5.9	0.2	15.6	0.4	5.6	0.1	12.4	0.2	26.4	0.7
CxL48D	0.5	0.0	8.2	0.1	18.6	3.7	5.0	0.1	16.2	0.2	26.0	1.1
CxL5H	1.2	0.0	7.0	0.3	13.9	1.1	3.8	0.2	11.5	0.1	34.8	0.9
CxL6E	1.8	0.0	10.3	0.6	16.8	3.1	5.7	0.3	19.5	2.3	26.6	1.1
CxL7a	1.6	0.5	9.7	0.4	18.3	6.1	9.9	0.5	19.5	1.9	29.7	5.4
CxL8I	1.8	0.1	8.1	0.2	16.2	1.0	5.1	0.1	16.9	0.2	31.9	0.8
Rialto	1.2	0.0	10.9	0.4	19.8	1.0	8.1	0.8	20.9	0.9	26.9	5.2
Average	**3.0**	**0.3**	**9.5**	**1.1**	**17.0**	**3.9**	**9.6**	**0.4**	**20.1**	**1.5**	**23.3**	**1.7**

Appendix Table A4.e. Genotype average for STB visual disease symptoms (%) on each leaf layer in the 2013 field experiment. Shown are disease scores in the untreated and fungicide-protected treatment at each disease assessment. Continued on the following page.

	GS59						GS59+14d						GS59+28d			
	L1		**L2**		**L3**		**L1**		**L2**		**L3**		**L1**		**L2**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**
Avalon	0.0	0.0	0.0	0.0	1.2	0.0	0.1	0.0	1.1	0.0	4.7	0.0	0.7	0.0	4.2	0.0
Badger	0.1	0.0	0.0	0.2	0.3	0.0	0.2	0.0	1.4	0.0	8.0	0.1	2.7	0.0	6.3	0.0
Battalion	0.0	0.0	0.0	0.3	0.9	0.0	0.2	0.0	0.9	0.0	7.0	0.0	1.2	0.0	4.7	0.0
Buster	0.0	0.0	0.0	0.1	0.7	0.0	0.2	0.0	1.0	0.0	9.7	0.0	7.6	0.0	11.6	0.0
Charger	0.0	0.0	0.0	0.1	1.3	0.0	0.4	0.0	2.5	0.0	18.6	0.0	2.6	0.0	8.3	0.0
Claire	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	1.1	0.0	6.0	0.3	0.6	0.0	5.9	0.0
Consort	0.0	0.0	0.0	0.0	2.2	0.1	0.1	0.0	1.9	0.1	15.3	0.0	2.5	0.0	16.5	0.0
L8xR 10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	1.3	0.3	0.1	0.1	0.4	0.0
L8xR 11	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.4	0.0	6.0	0.0	0.9	0.0	7.3	0.0
L8xR 14	0.0	0.0	0.0	0.2	1.2	0.0	1.1	0.0	2.0	0.0	16.9	0.0	3.0	0.0	8.1	0.0
L8xR 16	0.3	0.0	0.0	1.1	4.4	0.0	0.8	0.0	8.3	0.0	27.3	0.0	5.8	0.0	21.3	0.0
L8xR 20	0.0	0.0	0.0	0.5	1.3	0.0	0.5	0.0	2.9	0.0	11.5	0.0	2.0	0.1	15.4	0.0
L8xR 28	0.0	0.0	0.0	0.3	2.0	0.0	0.3	0.0	1.9	0.0	6.7	0.0	2.0	0.0	6.9	0.0
L8xR 34	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	6.3	0.0	0.7	0.0	1.7	0.0
L8xR 43	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.9	0.1	0.1	0.0	0.8	0.0
L8xR 44	0.0	0.0	0.0	0.1	0.7	0.0	0.1	0.0	4.2	0.0	5.0	0.0	2.0	0.0	10.2	0.0
L8xR 49	0.2	0.0	0.0	0.0	1.3	0.0	0.3	0.0	1.4	0.0	5.0	0.0	2.4	0.0	6.2	0.0
L8xR 51	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.6	0.0	3.4	0.0	0.5	0.0	4.0	0.0
L8xR 54	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.0	1.7	0.1	0.1	0.0	1.0	0.0
L8xR 57	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.8	0.0	0.3	0.0	2.5	0.0
L8xR 60	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.3	0.0	1.2	0.0	0.0	0.0	0.6	0.0
L8xR 64	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.4	0.0	2.2	0.0	0.5	0.0	2.5	0.0
L8xR 67	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.3	0.0	3.2	0.0	1.7	0.0	4.8	0.0
L8xR 71	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.0	0.6	0.0	3.4	0.0	0.9	0.0	3.1	0.0
L8xR 74	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.0	3.1	0.0	0.8	0.0	5.3	0.0
L8xR 80	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.6	0.0	6.9	0.0	0.2	0.0	5.6	0.0
L8xR 84	0.1	0.0	0.0	0.1	2.1	0.1	0.5	0.0	1.3	0.0	6.4	0.0	1.9	0.0	7.3	0.0

Appendix Table A4.e continued.

	GS59						GS59+14d						GS59+28d			
	L1		**L2**		**L3**		**L1**		**L2**		**L3**		**L1**		**L2**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**
CxL 48D	0.0	0.0	0.0	0.1	0.5	0.0	0.6	0.0	1.7	0.0	6.8	0.0	1.8	0.0	6.0	0.0
CxL 14B	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.3	0.0	8.7	0.0	0.8	0.0	2.8	0.0
CxL 3D	0.1	0.0	0.0	0.0	1.5	0.0	0.1	0.0	5.1	0.0	22.2	0.2	1.7	0.0	10.8	0.2
CxL 42C	0.0	0.0	0.0	0.3	1.8	0.0	0.5	0.0	2.6	0.0	8.4	0.1	3.7	0.0	10.0	0.0
CxL 5H	0.0	0.0	0.0	0.0	1.0	0.0	0.4	0.0	2.1	0.0	7.6	0.1	2.8	0.0	9.6	0.0
CxL 7A	0.0	0.0	0.0	0.6	1.3	0.0	0.4	0.0	1.0	0.0	3.1	0.0	3.2	0.0	5.7	0.0
Malacca	0.0	0.0	0.0	0.1	1.8	0.0	0.4	0.0	2.2	0.0	9.1	0.4	1.3	0.0	5.4	0.0
Moulin	0.0	0.0	0.0	0.1	1.3	0.0	0.1	0.0	2.1	0.0	9.7	0.0	2.6	0.0	6.4	0.0
Oakley	0.0	0.0	0.0	0.2	0.9	0.0	0.4	0.0	0.9	0.0	9.2	0.0	1.3	0.0	6.5	0.0
Option	0.0	0.0	0.0	0.0	1.7	0.0	1.5	0.0	1.3	0.0	5.9	0.0	2.6	0.0	6.5	0.0
Rialto	0.0	0.0	0.0	0.1	1.5	0.0	0.9	0.0	2.5	0.0	14.1	0.2	2.0	0.0	3.3	0.0
Savannah	0.0	0.0	0.0	0.2	1.6	0.0	0.2	0.0	2.9	0.1	26.7	0.0	3.0	0.0	13.8	0.0
Xi19	0.0	0.0	0.0	0.5	0.3	0.0	1.6	0.0	2.9	0.0	10.2	0.3	2.8	0.0	11.9	0.0
Average	0.0	0.0	0.0	0.1	1.0	0.0	0.3	0.0	1.6	0.0	8.3	0.1	1.9	0.0	6.7	0.0

Appendix Table A4.f. Genotype average for STB visual disease symptoms (%) on each leaf layer in the 2014 field experiment. Shown are disease scores in the untreated and fungicide-protected treatment at each disease assessment.

	GS59						GS59+14d						GS59+28d					
	L1		**L2**		**L3**		**L1**		**L2**		**L3**		**L1**		**L2**		**L3**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**
AxC 117	0.4	0.3	2.4	0.4	15.1	7.1	6.2	1.2	10.0	2.3	36.1	10.5	9.9	2.1	26.0	5.4	*	19.4
AxC 21	0.0	0.0	1.4	0.1	17.4	4.9	4.2	0.7	8.8	2.1	24.9	5.6	9.1	1.0	30.1	5.5	30.0	18.7
CxL 14B	0.0	0.2	0.3	0.1	3.9	0.4	0.7	0.5	2.2	0.8	11.9	3.8	1.5	0.6	11.3	3.7	25.5	21.8
CxL 43A	0.0	0.0	0.3	0.0	4.0	2.0	3.1	0.3	9.2	0.8	18.3	2.9	9.3	2.3	25.2	6.5	30.0	11.7
CxL 5H	0.3	0.2	0.4	0.2	3.5	0.4	1.9	0.3	5.3	0.9	24.5	3.7	4.6	0.3	35.8	2.6	*	16.4
CxL 7A	0.0	0.0	1.5	0.5	21.6	6.4	8.2	1.0	15.2	2.3	40.5	8.2	23.3	2.5	28.8	5.9	70.0	20.9
L14xR 114	0.0	0.0	0.7	0.4	6.5	2.3	2.9	1.1	6.9	2.6	18.4	7.9	7.4	3.9	25.6	12.6	39.4	30.1
L14xR 127	0.3	0.2	1.0	0.0	8.3	1.1	4.1	1.2	9.3	2.2	20.8	8.8	12.1	6.8	40.0	9.0	*	40.0
L14xR 133	0.0	0.2	0.7	1.0	8.9	2.5	3.7	1.0	8.7	1.7	25.1	6.8	10.4	1.0	31.7	7.8	55.0	16.9
L14xR 16	0.2	0.1	1.9	0.8	11.7	6.4	3.6	0.8	13.5	5.8	32.2	11.9	13.1	2.5	44.6	10.0	*	25.0
L14xR 19	0.0	0.2	10.3	0.8	37.8	5.0	12.7	1.6	39.3	5.3	46.3	17.7	32.4	4.8	48.8	14.9	70.0	41.0
L14xR 20	0.0	0.1	1.6	0.1	5.6	2.0	2.7	0.4	5.0	0.8	17.9	4.9	4.9	0.1	29.8	1.8	47.5	9.1
L14xR 22	0.2	0.0	3.2	0.7	16.3	4.9	11.3	1.9	42.9	4.0	65.0	27.2	22.9	7.6	20.0	17.8	*	20.0
L14xR 92	0.0	0.3	0.7	0.4	8.0	1.4	2.3	0.8	6.8	3.9	21.4	9.6	5.1	2.1	28.3	10.8	45.8	22.3
L8xR 10	0.0	0.0	0.5	0.2	5.8	4.6	1.6	0.1	11.7	1.5	21.5	14.1	3.8	0.2	22.1	7.8	50.9	35.0
L8xR 86	0.0	0.0	1.3	0.0	5.5	1.4	1.3	0.9	7.1	1.5	18.6	8.3	4.7	0.7	17.1	1.9	38.8	10.9
Grand Total	0.1	0.1	1.8	0.4	11.2	3.3	4.4	0.9	12.6	2.4	26.5	9.5	10.9	2.4	28.6	7.8	43.1	21.7

Appendix Table A4.g. Genotype average for green leaf area (%) on each leaf layer in the 2012 field experiment. Shown are GLA percentages in the untreated and fungicide-protected treatment at each disease assessment.

	GS59						GS59+14d						GS59+28d					
	L1		**L2**		**L3**		**L1**		**L2**		**L3**		**L1**		**L2**		**L3**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**
L14 x R 10	86.6	97.6	64.2	94.7	21.7	81.4	57.7	95.1	22.1	90.2	1.3	75.5	8.5	89.2	0.0	80.6	0.0	50.5
L14 x R 104	93.6	95.4	72.2	92.8	33.7	78.8	55.8	94.0	20.2	84.5	7.4	69.5	17.0	92.3	0.0	70.1	0.0	49.5
L14 x R 107	91.1	94.2	79.7	89.9	57.7	84.1	84.9	93.3	64.5	89.6	21.2	79.6	17.5	84.7	0.0	66.6	0.0	61.9
L14 x R 112	91.2	95.9	75.2	88.3	54.5	80.6	79.3	95.0	56.8	90.9	23.8	84.5	77.0	89.7	50.1	86.6	23.0	77.8
L14 x R 114	94.4	95.2	87.9	92.5	75.5	82.0	86.3	90.8	65.5	89.0	43.3	78.0	64.1	81.0	34.5	61.0	0.0	29.2
L14 x R 119	96.5	96.6	88.6	93.9	69.7	91.5	89.0	95.7	63.0	90.3	25.3	89.7	45.2	91.4	14.8	86.4	0.0	63.7
L14 x R 121	98.3	97.2	94.0	91.7	70.2	72.0	85.8	92.4	60.0	84.8	36.3	58.5	65.6	85.2	39.6	77.0	14.7	46.3
L14 x R 123	95.8	95.8	90.4	94.3	84.0	90.8	89.8	91.0	79.5	91.4	51.4	88.4	73.7	87.9	33.4	83.3	0.0	65.2
L14 x R 127	98.0	97.4	94.3	96.1	77.9	91.2	91.5	96.0	77.8	93.3	37.3	88.8	67.1	84.4	30.7	78.9	0.0	42.0
L14 x R 129	96.2	98.3	89.9	98.0	70.5	87.5	85.4	96.2	62.1	93.4	17.7	84.7	76.9	94.9	19.6	94.2	0.0	72.3
L14 x R 131	94.4	97.5	87.7	97.5	56.1	90.6	87.8	95.9	57.0	95.9	23.0	91.0	22.5	91.7	4.2	81.2	0.0	60.5
L14 x R 133	93.7	96.7	81.1	93.0	50.7	78.9	84.5	93.3	50.4	92.9	21.4	79.3	42.2	85.7	11.7	91.9	0.0	72.5
L14 x R 136	92.4	96.1	80.0	96.2	44.8	92.4	77.6	97.8	56.6	93.6	0.0	91.2	4.7	88.2	0.0	69.4	0.0	33.6
L14 x R 138	87.0	96.0	70.8	91.7	33.4	81.3	65.8	93.1	13.3	84.6	0.0	67.0	48.9	94.0	24.3	86.4	0.0	61.7
L14 x R 15	95.3	97.2	85.2	91.7	68.7	87.6	80.9	95.1	49.8	94.2	19.1	85.6	67.4	93.6	23.3	93.1	0.0	81.4
L14 x R 16	92.0	95.2	71.2	89.6	33.0	76.4	73.7	87.7	34.6	84.1	10.5	71.0	8.0	81.8	0.0	78.4	0.0	49.8
L14 x R 19	92.1	97.6	80.6	96.0	51.2	91.9	78.6	95.5	39.8	93.8	6.5	89.0	17.7	88.3	3.0	78.1	0.0	41.9
L14 x R 20	94.0	96.1	87.6	91.0	73.5	86.6	90.0	94.9	74.4	91.6	41.9	85.4	45.9	90.8	11.3	74.6	0.0	36.8
L14 x R 22	92.1	97.0	81.4	94.9	42.8	87.8	73.3	95.1	28.5	93.4	2.0	81.3	11.8	91.0	0.0	81.6	0.0	48.0
L14 x R 23	94.7	98.2	83.9	96.0	62.3	92.5	71.7	95.3	35.0	93.5	9.5	91.3	18.8	89.1	0.0	87.1	0.0	67.6
L14 x R 35	93.5	97.2	87.4	95.7	67.4	92.8	85.3	93.6	58.1	93.3	14.5	90.3	48.7	93.4	10.8	86.5	0.0	61.3
L14 x R 43	96.9	98.6	91.1	97.4	67.5	92.2	78.4	96.4	46.5	93.1	11.5	86.2	60.5	95.3	11.0	93.5	0.0	85.4
L14 x R 5	93.3	97.9	78.6	93.7	60.7	80.6	75.8	94.7	32.8	87.5	16.3	80.8	64.2	93.7	16.1	88.3	0.0	63.1
L14 x R 50	92.7	93.4	69.0	90.0	40.9	81.3	73.0	93.1	43.2	86.6	17.7	69.7	49.3	94.1	16.1	87.4	0.0	68.5
L14 x R 55	92.8	97.5	80.5	95.0	42.7	83.7	81.9	94.2	40.3	91.9	7.7	77.9	21.5	91.9	0.0	88.2	0.0	56.5
L14 x R 59	97.0	98.1	89.1	91.4	65.3	72.7	83.5	93.0	56.3	85.2	21.8	70.3	72.6	92.2	26.8	85.2	0.0	61.6

Appendix Table A4.g continued.

	GS59						GS59+14d						GS59+28d					
	L1		**L2**		**L3**		**L1**		**L2**		**L3**		**L1**		**L2**		**L3**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**
L14 x R 62	92.6	97.2	65.9	86.6	24.8	70.9	62.8	93.5	19.0	83.8	2.3	50.3	24.0	92.1	4.8	78.9	0.0	52.1
L14 x R 66	93.5	95.0	81.5	91.3	57.1	87.3	85.7	94.3	63.7	90.0	17.2	89.2	31.1	85.1	5.2	81.7	0.0	48.7
L14 x R 68	95.9	96.8	92.8	96.8	80.6	96.0	90.6	96.9	80.6	97.2	59.5	94.3	66.5	94.7	20.4	93.0	0.0	71.2
L14 x R 7	92.9	94.2	74.7	83.7	44.9	72.9	74.7	91.3	43.3	83.9	13.7	66.8	38.0	86.5	2.7	73.7	0.0	22.0
L14 x R 70	90.2	94.3	80.2	95.9	60.8	89.5	83.1	96.1	58.7	94.7	22.5	92.7	23.2	93.0	7.8	84.1	0.0	54.8
L14 x R 74	93.4	95.9	76.9	91.3	67.8	77.6	76.7	94.0	45.7	88.1	17.5	76.4	49.3	94.0	13.3	87.1	7.0	67.8
L14 x R 80	97.2	97.0	90.2	91.5	72.9	78.2	82.0	92.8	57.6	87.0	26.0	77.0	74.3	91.0	37.2	86.5	9.1	76.8
L14 x R 82	87.6	96.7	75.8	95.6	58.7	94.6	84.8	95.8	60.4	94.6	30.4	87.8	14.3	87.8	0.0	81.9	0.0	57.0
L14 x R 85	94.9	98.2	84.4	97.0	63.2	96.1	87.9	95.9	63.3	95.8	15.5	92.6	39.0	93.3	4.5	91.8	0.0	72.3
L14 x R 89	98.0	98.3	93.3	96.8	77.4	94.2	85.3	94.3	57.4	89.6	22.4	85.0	70.0	90.4	20.0	91.5	0.0	80.0
L14 x R 91	89.3	92.7	77.1	82.0	47.8	66.9	69.0	93.6	25.1	76.7	5.7	61.3	9.2	83.5	0.0	49.6	0.0	27.7
L14 x R 92	97.0	97.1	93.1	93.7	81.4	84.7	90.4	92.8	74.6	88.9	57.0	81.4	78.6	86.4	34.7	69.2	10.0	61.2
CxL 14B	94.1	93.7	90.4	94.3	74.6	91.4	90.6	93.6	81.3	92.7	52.7	83.2	57.4	83.6	28.3	67.3	0.0	49.9
CxL 3D	95.5	96.8	86.4	95.6	68.3	89.7	89.8	95.9	75.6	95.5	17.3	93.0	28.9	93.4	0.0	87.7	0.0	44.6
CxL 41F	93.0	97.2	84.0	97.1	51.7	93.7	85.4	96.8	59.8	96.4	21.7	91.0	26.8	91.3	0.0	81.2	0.0	46.6
CxL 42C	93.4	95.0	81.6	92.0	50.8	85.2	80.3	92.8	50.5	89.1	16.5	81.2	25.9	87.1	0.0	79.2	0.0	39.5
CxL 43A	95.4	96.8	89.5	97.4	69.2	96.5	88.8	95.7	66.3	95.4	24.0	91.0	50.8	93.1	0.0	90.4	0.0	63.8
CxL 48D	95.7	95.9	88.1	95.8	56.6	90.4	88.7	95.2	63.3	95.6	9.7	90.0	34.7	89.9	3.2	85.3	0.0	44.8
CxL 5H	95.9	97.6	89.4	97.2	74.6	92.0	92.2	97.2	74.2	98.0	35.6	95.9	46.2	95.4	8.3	92.3	0.0	50.9
CxL 6E	96.2	97.6	83.0	97.0	62.6	93.1	87.0	94.8	67.7	93.9	26.2	92.1	43.7	93.5	6.2	93.4	0.0	75.5
CxL 7a	95.7	97.0	80.3	92.9	57.5	72.9	86.4	96.5	58.1	88.9	9.1	60.2	12.4	85.5	0.0	72.2	0.0	13.0
CxL 8I	93.5	97.1	83.3	96.0	58.0	83.6	88.7	95.3	60.3	94.9	13.2	90.0	41.4	92.9	0.0	90.4	0.0	62.3
Rialto	95.9	96.9	86.2	96.7	61.0	95.9	87.5	96.0	70.7	95.7	19.9	91.8	25.8	87.2	2.0	84.2	0.0	62.5
Average	**93.9**	**96.5**	**83.1**	**93.6**	**59.1**	**85.8**	**81.7**	**94.5**	**54.4**	**91.0**	**20.9**	**82.0**	**41.4**	**89.9**	**11.8**	**82.2**	**1.3**	**56.2**

Appendix Table A4.h. Genotype average for green leaf area (%) on each leaf layer in the 2013 field experiment. Shown are GLA percentages in the untreated and fungicide-protected treatment at each disease assessment. Table continued on the following page.

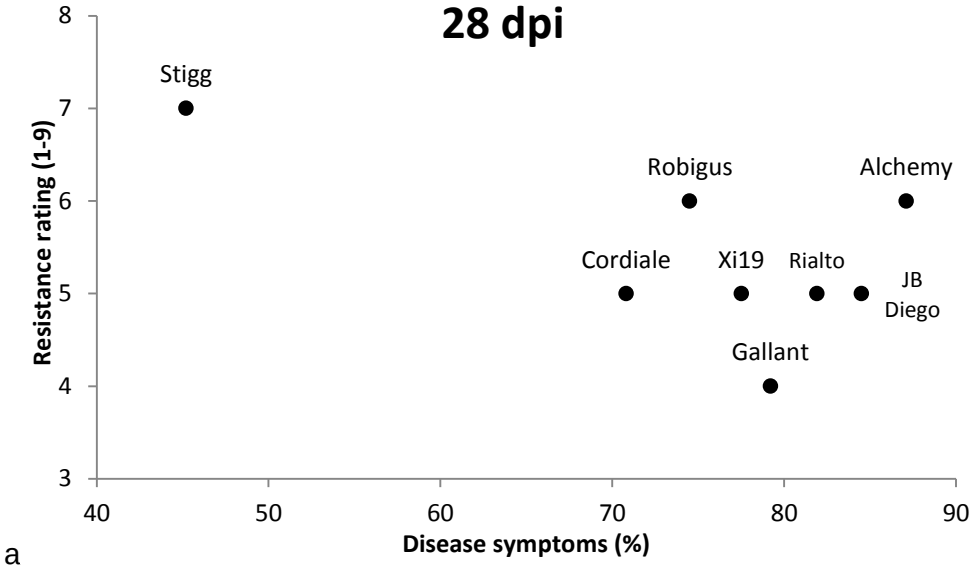
	GS59						GS59+14d						GS59+28d					
	L1		**L2**		**L3**		**L1**		**L2**		**L3**		**L1**		**L2**		**L3**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**
Avalon	98.5	98.2	97.9	98.1	96.1	96.9	97.6	97.5	96.7	98.1	88.5	93.8	89.6	80.7	46.3	39.5	0.0	3.1
Badger	98.7	98.6	98.2	98.1	97.5	97.7	96.8	97.1	93.0	97.5	59.1	87.0	60.5	75.5	13.3	21.7	0.0	0.0
Battalion	98.4	97.8	98.3	98.2	97.7	97.5	94.6	96.2	88.9	91.4	62.9	75.0	66.1	77.6	35.1	33.9	0.0	3.3
Buster	98.1	98.3	98.6	98.4	96.7	97.7	94.3	97.3	94.6	97.4	79.9	89.5	73.7	72.4	31.8	41.4	0.0	3.3
Charger	98.0	98.6	98.1	98.5	92.0	97.7	95.1	95.7	95.3	96.5	73.8	78.2	58.2	77.0	7.8	29.0	0.0	1.5
Claire	98.6	98.6	98.5	98.7	97.8	98.1	97.4	97.1	94.7	97.7	72.2	94.8	72.9	79.1	18.0	31.9	0.0	0.0
Consort	97.9	98.1	98.5	98.5	95.2	96.2	95.8	95.2	93.0	91.0	67.8	75.6	75.5	63.2	24.7	36.6	0.0	4.2
L8xR 10	99.1	98.9	98.8	98.3	97.4	96.2	96.9	97.5	96.3	97.1	91.6	93.2	82.0	88.8	50.9	60.6	13.2	15.5
L8xR 11	97.5	97.5	97.2	97.8	91.6	94.6	93.5	95.5	95.2	96.2	88.2	94.6	70.0	82.8	63.7	73.4	20.7	34.5
L8xR 14	98.5	98.5	97.7	97.8	95.0	95.1	96.9	94.3	96.2	96.2	77.5	90.5	61.2	86.2	39.9	67.4	0.0	44.6
L8xR 16	98.3	98.6	97.1	98.5	92.5	95.7	96.9	97.2	89.6	96.1	64.8	90.5	86.1	88.9	40.9	81.2	4.0	25.2
L8xR 20	98.6	98.4	97.2	98.1	94.1	95.6	91.2	95.4	89.1	96.1	76.9	94.7	60.2	59.5	39.7	54.2	0.0	12.2
L8xR 28	98.8	98.1	98.1	98.4	94.6	95.8	98.0	97.6	95.7	97.7	74.8	93.2	59.1	72.7	24.2	53.2	0.0	0.0
L8xR 34	98.6	98.3	97.9	97.5	94.9	96.8	95.9	95.6	95.0	96.3	85.2	91.7	82.7	80.4	71.0	62.7	16.2	10.7
L8xR 43	98.5	98.6	98.3	98.2	97.0	96.8	98.3	97.9	98.1	98.2	94.3	96.4	61.4	82.3	33.0	63.3	2.8	8.2
L8xR 44	98.7	98.2	97.7	91.3	94.6	80.0	97.7	93.3	92.6	88.3	81.1	73.4	52.0	45.7	28.5	34.7	6.8	0.0
L8xR 49	98.8	98.6	98.9	98.5	96.9	97.5	98.2	98.2	97.0	98.2	86.9	97.8	69.4	82.4	56.8	76.0	16.9	22.4
L8xR 51	98.6	98.5	98.7	98.1	96.8	95.7	95.7	96.3	94.0	94.4	90.4	93.6	82.9	82.4	52.4	46.7	8.0	0.0
L8xR 54	98.9	98.7	98.5	98.4	97.8	97.0	97.8	96.6	97.4	97.2	95.0	93.2	90.5	67.1	61.0	38.0	14.7	27.0
L8xR 57	98.7	98.8	98.4	98.4	97.6	96.7	96.0	97.3	95.5	98.1	93.4	96.6	64.7	75.2	60.8	69.4	8.4	17.8
L8xR 60	98.6	98.1	98.1	96.8	95.4	94.2	96.9	96.5	96.2	95.9	91.8	92.5	82.8	66.3	71.8	45.7	22.6	22.2
L8xR 64	98.8	98.9	98.6	98.8	97.3	97.2	97.5	97.7	94.6	98.0	91.4	94.9	63.8	72.4	51.1	42.8	6.7	8.0
L8xR 67	98.6	98.8	98.6	98.5	96.1	96.1	97.8	97.2	97.5	97.5	91.3	94.2	63.6	67.4	30.5	40.2	0.0	20.7
L8xR 71	98.8	98.9	98.7	98.6	96.2	98.0	97.6	96.7	97.6	94.2	91.4	92.8	66.7	73.8	40.6	45.3	16.5	20.1
L8xR 74	98.4	98.6	98.7	98.4	97.8	96.9	95.1	95.6	94.9	95.3	86.4	88.4	66.4	67.7	39.5	47.1	4.2	0.0
L8xR 80	98.7	98.6	98.0	98.6	95.9	94.1	97.1	97.8	95.9	97.7	70.2	89.0	39.6	67.0	17.5	48.9	0.0	11.5
L8xR 84	98.5	98.4	97.9	98.7	96.2	97.7	96.3	97.2	96.4	97.5	91.7	96.3	90.7	88.8	75.5	90.7	19.3	47.1

Appendix Table A4.h continued.

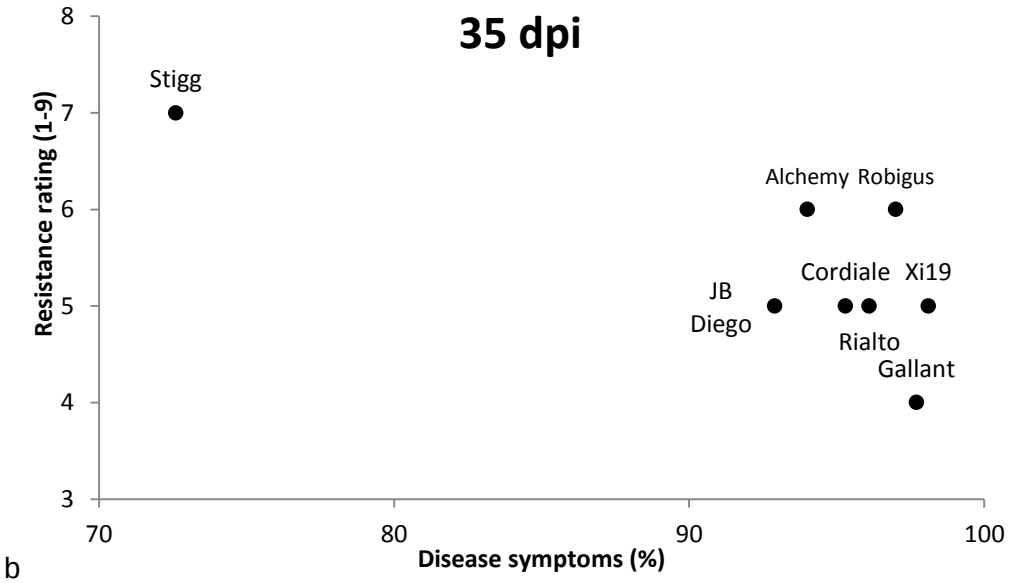
	GS59						GS59+14d						GS59+28d					
	L1		L2		L3		L1		L2		L3		L1		L2		L3	
Genotype	Untr.	Prot	Untr	Prot	Untr	Prot	Untr.	Prot	Untr	Prot	Untr	Prot	Untr.	Prot	Untr	Prot	Untr	Prot
CxL 48D	97.9	98.1	98.1	97.6	96.1	95.2	94.8	94.8	92.9	95.2	76.7	90.4	68.3	72.2	27.3	22.0	0.0	2.9
CxL 14B	97.2	98.1	97.7	98.1	96.9	97.1	96.1	96.4	95.9	96.5	80.1	95.2	63.1	71.1	24.0	48.7	0.0	7.8
CxL 3D	98.5	98.6	98.7	98.3	95.7	97.7	96.9	96.2	92.6	93.4	60.7	86.6	71.1	67.0	27.7	39.1	0.0	0.0
CxL 42C	97.6	98.3	98.1	98.2	94.5	96.1	94.2	97.4	84.4	95.9	54.4	82.2	63.2	78.9	13.2	30.9	0.0	0.0
CxL 5H	98.0	97.8	97.9	97.9	96.3	95.7	95.7	95.2	94.6	90.5	64.3	76.9	56.5	67.3	10.2	27.7	0.0	0.0
CxL 7A	98.4	98.2	98.1	98.1	96.6	96.4	95.5	96.7	92.8	96.3	84.1	92.4	70.0	80.8	22.8	48.0	3.3	3.2
Malacca	97.6	97.7	98.2	97.5	95.3	96.6	93.2	94.8	89.8	96.4	65.0	89.9	56.4	67.2	10.7	31.5	0.0	1.8
Moulin	98.3	98.0	98.5	98.6	96.9	97.2	97.2	96.3	95.9	93.9	76.4	74.1	67.6	73.1	15.0	26.4	0.0	0.0
Oakley	98.8	98.6	98.6	98.7	97.1	97.5	96.4	96.6	95.3	96.9	60.4	92.6	72.7	68.5	22.6	31.2	0.0	0.0
Option	98.0	98.0	98.2	97.9	95.4	96.0	93.7	96.1	96.3	96.3	83.3	90.3	56.3	70.0	23.1	36.9	0.0	3.7
Rialto	98.7	98.4	98.4	98.7	96.4	98.6	95.9	94.4	91.7	98.1	74.0	96.1	67.0	82.5	35.9	54.6	1.5	2.7
Savannah	98.2	98.3	98.2	98.4	95.3	97.6	96.1	97.2	93.9	95.8	56.3	83.5	77.2	69.9	25.5	39.6	0.0	5.0
Xi19	98.9	98.8	98.4	98.6	97.7	97.6	96.2	97.6	92.7	98.2	81.8	89.0	76.8	69.1	17.0	20.2	0.0	2.3
Average	**98.4**	**98.4**	**98.2**	**98.1**	**96.0**	**96.2**	**96.1**	**96.4**	**94.2**	**96.0**	**78.4**	**89.5**	**69.0**	**74.0**	**35.0**	**45.8**	**4.6**	**9.8**

Appendix Table A4.i. Genotype average for green leaf area (%) on each leaf layer in the 2014 field experiment. Shown are GLA percentages in the untreated and fungicide-protected treatment at each disease assessment.

	GS59						GS59+14d						GS59+28d					
	L1		**L2**		**L3**		**L1**		**L2**		**L3**		**L1**		**L2**		**L3**	
Genotype	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**	**Untr.**	**Prot**	**Untr**	**Prot**	**Untr**	**Prot**
AxC 117	96.5	97.6	91.3	96.4	73.3	88.5	91.3	96.5	85.8	94.2	48.3	84.3	84.6	92.5	35.8	89.5	0.0	64.6
AxC 21	95.4	97.6	94.0	97.3	73.8	89.4	92.9	96.7	85.8	94.0	55.2	89.3	83.8	95.2	45.4	89.2	5.0	62.1
CxL 14B	96.3	97.6	95.3	96.9	90.8	96.0	94.7	96.0	91.8	94.9	76.2	92.0	83.3	88.3	76.3	85.8	15.0	67.5
CxL 43A	97.3	98.3	88.9	97.5	89.8	94.2	94.7	97.3	86.3	96.7	65.6	93.8	86.3	92.9	62.5	86.7	4.2	78.8
CxL 5H	96.8	97.9	94.9	97.7	91.9	97.1	95.9	97.3	90.5	96.6	62.9	92.6	90.4	95.4	45.0	92.5	0.0	69.2
CxL 7A	97.2	98.2	94.7	97.4	67.9	88.1	88.3	95.7	80.1	94.8	42.5	87.5	61.7	93.8	18.3	89.8	0.8	63.8
L14xR 114	95.8	96.8	93.7	96.1	85.0	93.5	93.3	96.1	89.1	94.1	73.3	88.3	82.5	89.2	65.4	81.3	16.7	45.6
L14xR 127	94.4	96.3	95.8	97.5	84.2	95.4	91.3	95.6	85.7	93.8	64.2	85.8	45.4	65.6	22.9	62.9	0.0	11.7
L14xR 133	97.1	97.6	94.2	95.5	83.9	93.5	87.1	95.5	82.3	94.4	62.9	89.5	72.9	90.3	37.9	85.0	3.3	73.8
L14xR 16	97.6	97.2	94.3	95.9	80.0	87.6	93.4	96.2	80.6	90.8	37.5	81.2	80.0	92.3	26.7	84.6	0.0	47.1
L14xR 19	72.9	96.8	75.8	96.8	40.0	91.1	71.6	94.9	44.2	90.5	12.1	74.6	37.9	90.0	11.7	75.0	1.7	19.2
L14xR 20	97.0	97.8	93.8	97.3	84.4	95.0	93.6	96.3	91.5	96.5	77.1	91.6	87.1	95.2	56.3	92.9	10.0	75.8
L14xR 22	97.3	98.1	93.3	96.8	70.0	92.2	84.2	96.2	46.7	92.8	5.0	61.7	37.1	83.8	5.8	67.9	0.0	5.8
L14xR 92	96.3	97.3	93.3	96.3	85.4	94.4	94.4	96.5	89.7	92.8	62.3	85.0	82.5	90.8	61.7	83.3	15.4	67.9
L8xR 10	95.3	97.7	94.2	96.8	85.2	91.5	93.1	96.4	77.4	94.9	60.0	77.8	84.2	90.3	62.5	82.1	13.3	47.5
L8xR 86	96.7	97.3	94.8	97.1	87.3	95.2	95.7	96.0	89.3	95.3	75.6	86.6	89.6	93.3	75.4	91.3	6.7	73.3
Average	95.0	97.5	92.6	96.8	79.5	92.7	90.9	96.2	81.0	94.2	55.0	85.1	74.3	89.9	44.3	83.7	5.8	54.6



a



b

Appendix Fig A6.1. Average disease symptoms of the commercial genotypes and the relationship with recommended list and producer resistance ratings at a) 28 days post inoculation and b) 35 days post inoculation.

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