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Investigations into the characteristics of historic barley varieties with reference to fungal diseases and physiology

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Abstract

The aim of this study was to characterise modern and historic barley varieties for agronomic and growth characteristics and to assess their resistance to *Fusarium* and mildew diseases.

Barley is a major agricultural crop cultivated throughout the world providing an important source of energy and protein for humans and animals. To achieve its potential, however, it must be carefully managed to avoid diseases particularly those caused by fungi which can cause serious economic losses and affect food safety and quality.

Contemporary barley varieties have been selected for yield and disease resistance. However, long term resistance to disease is increasingly difficult to achieve as microorganisms mutate and maintain their virulence. Investigating the potential of historic barley varieties as a genetic resource for future developments is one approach to obtaining novel attributes which may have been overlooked when breeding focused on yield rather than character of barley and on disease resistance.

To examine the characteristics and disease resistance of historic barley varieties a series of investigations was conducted. Initially a screening was initiated by growing thirteen historic barley varieties and two modern barley varieties in a field trial in 2009. Growth features, yield and symptoms of mildew and Fusarium Head Blight (FHB) were scored and compared. This field experiment was repeated in 2010 with six of these varieties at the John Innes Centre by deliberately exposing the plants to *F. culmorum Fu 42*. A further experiment was conducted at the same time by growing seven varieties in glasshouse conditions at the University of Sunderland under inoculated and uninoculated conditions.

From both growing seasons clear differences were found for the level of *F. culmorum* infection between the different barley cultivars with infection levels in heads ranging from 16% for Chevalier and 86.4% for Tipple barley varieties respectively. Nitrogen increased the level of FHB in all varieties possibly because

of increased plant leaf number, tillers and humidity within the environment around the plant.

Mycotoxin analysis showed that *F. culmorum* infection resulted in mycotoxin contamination of all varieties. However, levels of mycotoxin were significantly lower in Chevalier barley compared to other barley varieties including the two modern varieties, Tipple and Westminster. Observations using scanning electron microscopy indicated a different pattern of fungal growth in Chevalier barley with limited fungal development on both external and internal surfaces compared to other susceptible varieties.

In general resistance against FHB disease depends on variable responses including plant physiology and morphology, antifungal compounds or resistance genes. Different flowering dates or flowering periods could be also considered reasons for different infection levels. However, in this study the duration of anthesis was not assessed and could be an important factor. Further experiments to identify the flowering times of different varieties could be considered for further research.

The lower levels of disease associated with lower levels of mycotoxins and a reduced fungal development in Chevalier barley indicated that this variety has a strong resistance against FHB disease. This may be because of its late flowering and its tall height minimising colonisation from the soil. However, Chevalier barley was found to be very susceptible to powdery mildew disease, particularly in glasshouse studies.

The potential of Chevalier barley to produce good malt was indicated when compared to modern varieties suggesting that Chevalier may be a valuable breeding stock for future developments.

Nafferton farm

JIC farm

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List of Abbreviations and Symbols.

ANOVA	Analysis of variance
AUDPC	Area under the disease progress curve
BBCSRC	Biotechnology and biological Sciences Research Council
Bgh	*Blumeria graminis* f. sp*. Hordei*
BYDV	Barley yellow dwarf virus
CV	Coefficient of variation
DON	Deoxynivalenol
Dp	Diastatic power
ELISA	Enzyme-linked immunosorbent assay
FHB	Fusarium head blight
FLD	Fluorescenic detection
FPE	Fusarium protein equivalent
GA	Gibberellic acid
GC	Gas chromatography
GE	Germination energy
GS	Growth stage
ha	Hectare
HGCA	Home-Grown Cereals Authority
HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography
HPLC/MS	High-performance liquid chromatography mass spectrometric
HR	Hypersensitivity

JIC	John Innes Canter
K	Potassium
L	Levenes test
LSD	Least significant difference
N	Nitrogen
NIL	Near-isogenic line
NIV	Nivalenol
NP	Non parametric test
P	Phosphorus
P	probability
PBS	Phosphate-buffered saline
PCR	polymerase chain reaction
PDA	Potato dextrose agar
ppb	Part per billion
PSH	*Puccinia striiformis* f. sp*. Hordei*
QTL	Quantitative trait locus
ROSA	Rapid One Step Assay
SEM	Scanning electron microscopy
sdw1	Semi-dwarf gene
SMM	S methyl methionine
UV	Ultraviolet
USDA	United States Department of Agriculture

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Chapter one

General introduction

1.1 Barley as a crop.

Barley (*Hordeum vulgare*) belongs to the grass family Gramineae and grows in many countries, generally in temperate regions. Barley is one of the most important cereal crops worldwide and the fourth most essential cereal after wheat, rice and maize (Winch, 2007) and has a wide range of cultivation in diverse sites and temperatures (Ullrich, 2011). It is able to grow well vegetatively under cold conditions and also has the ability to grow under very hot weather during and after heading (Winch, 2007). It was domesticated early and used for many different purposes including food for humans and animals, for malting to produce malt beverages and for numerous medicinal purposes since ancient times. Barley's growing season is short, and has an early maturation with the capability of a high yield production of 6 tonnes per hectare (ha) (Vaughan *et al*, 2009; Sun and Gong, 2010). Winter barley varieties need more than 180 days to reach maturity whereas spring varieties need about 85-120 days (Winch, 2007).

Badr *et al* (2000) revealed that the Israel-Jordan area is the district where wild barley was domesticated. However, the cultivation of barley was possibly initiated in the highlands of Ethiopia and South-east Asia where it was the principal bread plant for Hebrews, Greek and Romans (Winch, 2007) and has been cultivated in Syria since 1300 BC. The Romans obtained barley from Egypt and other parts of Africa and Spain (Johnson and Emerson, 1851). Currently barley is extensively grown in most European countries, America and in temperate regions of Asia and Africa, as well as other hotter and drier areas (Winch, 2007).

Barley requires less fertile soil and adapts to a wide range of soil types compared to wheat. It is more salt tolerant, ripens earlier and is more drought resistant compared to wheat. The optimal temperature for germination and emergence is 15-20°C and the minimum temperature is 2°C (Winch, 2007).

Barley contains beneficial components which are reportedly good for health and include fibre, antioxidants, phytochemicals, vitamins and minerals (Swenson, 2008). For example, barley β-glucan has been shown to reduce blood lipids (Keenan *et al*, 2007).

The barley plant grows through nine major growth phases: Seedling stage, leaf emergence, tillering, stem elongation, booting stage, flowering, ear formation, grain filling and ripening (Figure 1.1) (Tottman and Makepeace, 1979). Each phase has more detailed growth stages identified in relation to physiological developments. These stages are numerically assigned from GS10, “first leaf through coleoptile”, to GS92, “grain hard”. Environmental and management factors acting at different stages have varying effects on the final yield of grain at harvest. Management is targeted to maximise growth on the stages which most influence yield.

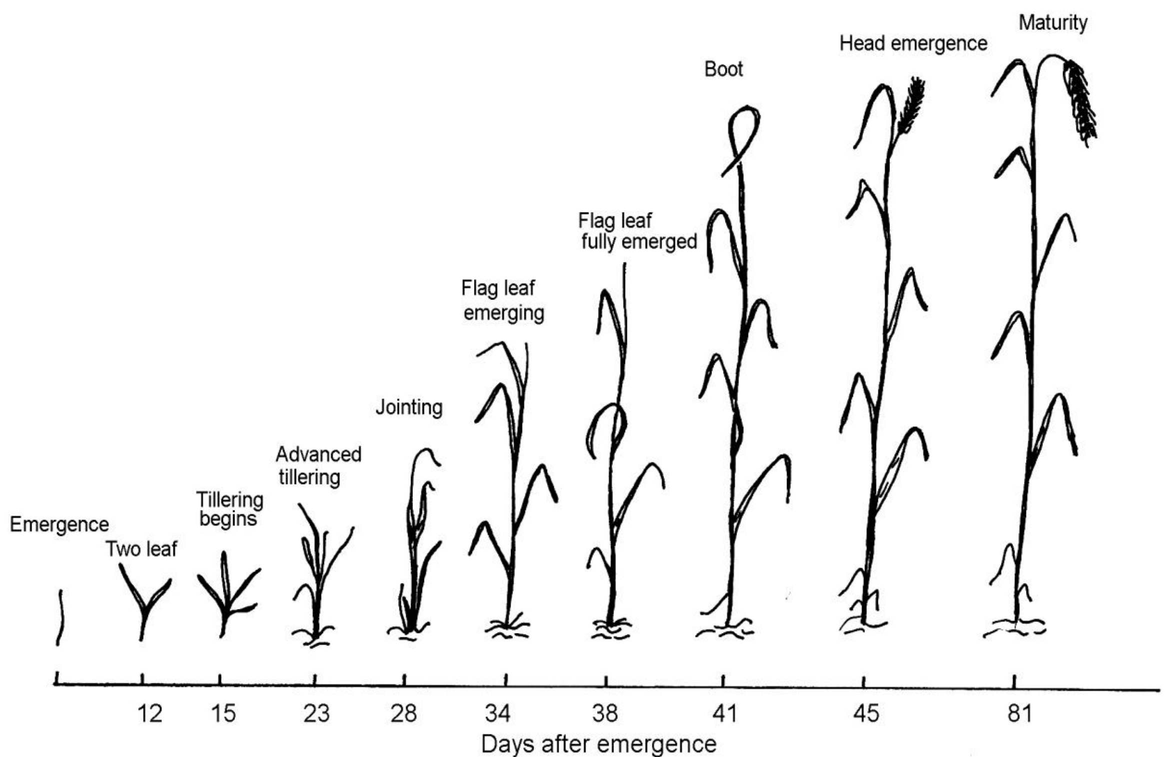


Figure 1.1: Major growth stages of spring barley (drawn after Tottman and Makepeace, 1979).

Temperature and photoperiod are major factors governing the speed of barley development and account for different rates of development in different locations. The effect of temperature on germination and emergence is particularly relevant and is measured in thermal time– the ^{o}C days of accumulated mean daily temperature from sowing. As a reference 150°C days are taken as a target for 50% emergence (Tottman and Makepeace, 1979).

The growth habit of barley is designated either winter or spring while the grains on the head are either two-row or six-row. Winter barley varieties are sown in autumn for harvest in the following July while spring barley varieties are sown between December and April for harvest between August and September. A greater yield of approximately 20% is typically obtained from winter sown varieties but at a greater cost of the longer growth period. Typical yields are around 15 tonnes dry weight per hectare at harvest with around 50% as grain yield.

The effect of overwintering requires winter barley varieties to have a high winter hardiness rating. Spring barley varieties are less hardy and are more dependent on site conditions and good management to achieve target yields than winter varieties which have more opportunity to compensate for deficiencies. Barley has less potential to compensate for losses compared to wheat as it only produces one floret per spikelet whereas wheat may increase ear size and number per plant.

Barley grains form spikelets on the central stem or rachis of the ear. The ovary of each spikelet is sheathed by a lemma and a palea which makes the flower in most two-row cleistogamous and although the ovary is self-fertilized stamens are produced (Figure 1.2). Three spikelet's form at each node along the rachis and alternate in direction producing up to 24 grains per ear (Tottman and Makepeace, 1979).

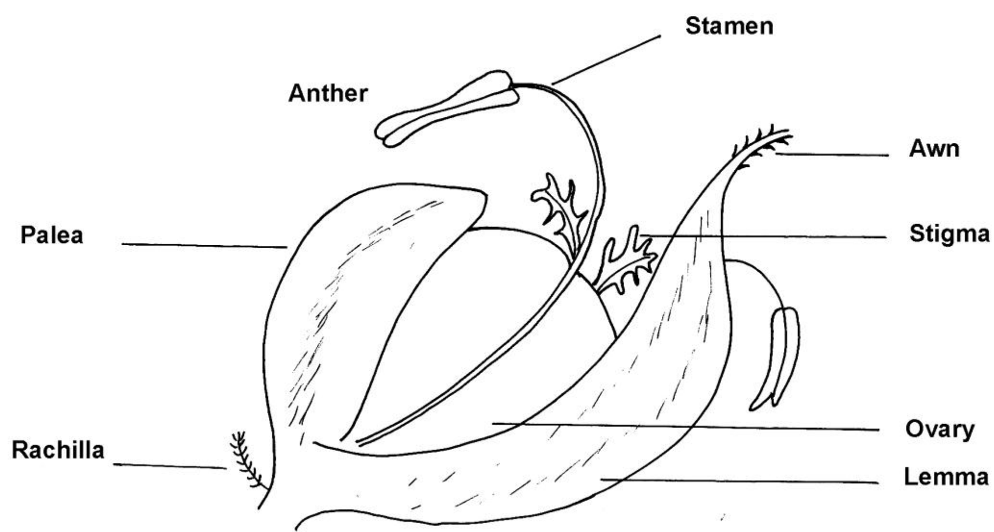


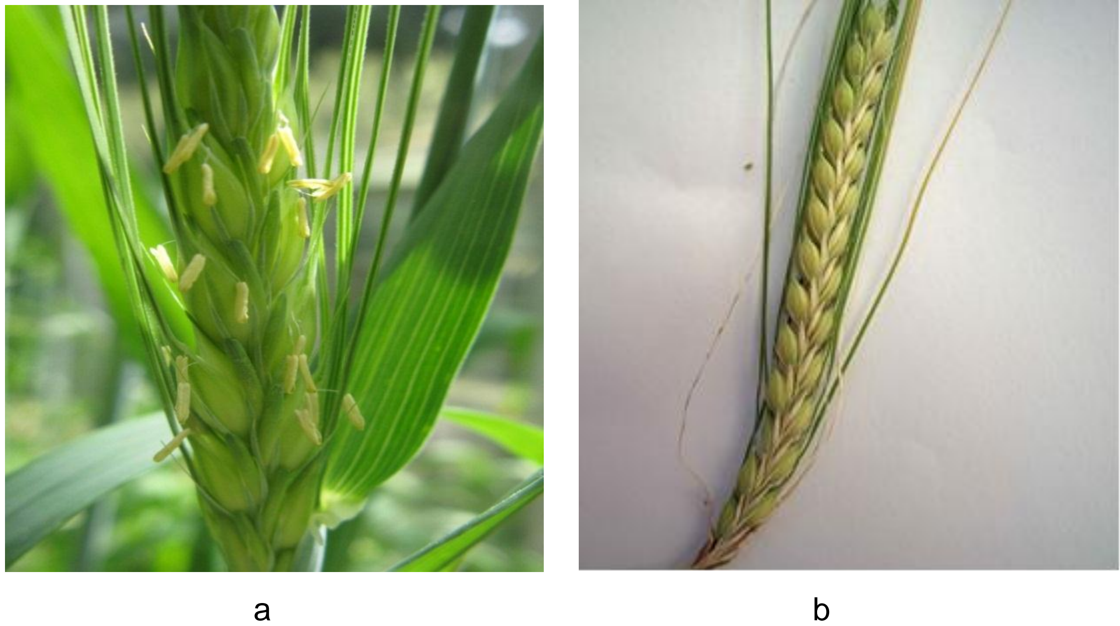
Figure 1.2: Barley spikelet features.

Barley varieties differ in many features from dormancy and tillering to grain filling and response to soil nitrogen. A continual drive to develop higher yields and productivity has produced many hundreds of varieties with many different features and selected genetics. One major differentiation is that between six-row and two-row varieties. In six-row varieties all three spikelets are fertilized at each node whereas in two-row varieties only one spikelet is fertilized. Grains from six-row varieties tend to be smaller and have higher levels of protein and enzymes than grains from two-row varieties which are larger and with less protein.

In wild barley, the two-row phenotype is regarded as the ancestral form which was believed to be transformed to a six-row head by mutation during domestication. Genetic studies have revealed the conversion is attributed to a mutation in *vrs1* gene located on chromosome 2HL (Pourkheirandish and Komatsuda, 2007).

After fertilization energy reserves are redistributed from stem to grains which fill and ripen over a 20 day period producing a final dry weight of around 40 mg per grain. Examples of field grown barley and seed heads are shown in Figures 1.3 and 1.4.

Figure 1.3: Field grown barley.



a b

Figure 1.4: a- six-row barley head b- two-row barley head.

1.2 Malting barley and desired characteristics.

Barley is the elementary raw material for producing malt beverages in addition to numerous food products (Raulio *et al*, 2009). Different barley varieties are recommended for malting in different countries. The annual UK barley production is around 6.5 million tonnes per annum, 2 million tonnes are used by the home malting industry and an extra 100,000-400,000 tonnes of malting barley are exported each year (HGCA, 2001). Achieving the specifications of malt production is a technical challenge which is still being refined and which depends strongly on barley variety used.

The malting process comprises three main steps, soaking or steeping to increase barley water content, germination to modify the endosperm and aggregate hydrolytic enzymes and drying or kilning to stop germination and develop colour and flavours (Allosio-Ouarnier *et al*, 2000). The rootlets produced during germination are removed by sieving in order to leave finished malt ready for use (HGCA, 2001).

The quality of malting barley depends on features such as grain size and weight (large grains generally have a higher starch concentration which increases the

efficiency of the extracting purposes), size distribution, germination, enzyme activity, protein levels and nitrogen levels (Horsley *et al*, 2009). In Europe, two-row barley is favoured more than six-row cultivars for malting purposes because six-row barley cultivars tend to have more variation in grain size and higher protein concentration (Riggs and Kirby, 1978). Large grains from two-row barleys modify more evenly in malting and suit isothermal mashing systems.

Malting barley should ideally contain 9.5 -11.5% protein, low levels of nitrogen between 1.55 and 1.75% (usually malting barleys contain lower levels of nitrogen compared to feed barleys) and have a grain germination rate >95% (Thylen *et al*, 1999; HGCA, 2001).

The concentration of nitrogen in the grain is an important factor determining malt quality and market price. For malting purposes, nitrogen application rates are recommended to be adjusted for grain nitrogen concentration rather than for maximum yield (Welsh *et al*, 2003).

The recommended malting barley varieties in United Kingdom, United State and Canada for 2010/2011 are shown in Table 1.1.

Table 1.1: Recommended malting barley varieties in United Kingdom, United State and Canada for 2010/2011 (HGCA, 2011; USDA, 2011; Canadian Grain Commission, 2011).

UK	USA		Canada	
Propino	AC Metcalfe	Lacey	AC	Robust
Panther	CDC Copeland	Legacy	Metcalfe	CDC Yorkton
Quench	Charles	Rasmusson	Copeland	CDC Battleford
Shuffle	Conlon	Robust	Newdale	
Concerto	Conrad	Stellar-ND	Polarstar	
Moonshine	Harrington	Tradition	CDC	
NFC-Tipple	Hockett		Kendall	
Publican	Merit		Harrington	
Forensic	Merit 57		Merit	
Belgravia	Moravian 37		Meredith	
Westminster	Moravian 69		Other	
Oxbridge	Scarlett		Lacey	
Optic	Drummond			

1.3 Microbes contaminating barley in storage.

Barley grains are stored between two months to one year to allow the breakup of the normal dormancy before malting. However, through the storage period barley grains are often exposed to different levels of temperatures and humidity which increase the grains susceptibility to storage microbes and pests (Magan and Lacey, 1988). The composition of the microbial community on barley grains changes dramatically after harvest as a result of post-harvest operations as detailed in Table 1.2. In general microbial growth and community are higher throughout the germination period, however the number of microbes drop after kilning. Bacteria are the dominant microbes while the yeast and fungi community decreases gradually (Petters *et al*, 1988).

Table 1.2: Microbes attacking barley during storage and processing (Flannigan, 2003).

Directly after harvest	After storage	After steeping	Early hours of kilning
Alernaria	*Penicillium*	*Fusarium*	*Rhizopus*
Stemphylium	*Aspergillus*	*Rhizopus*	*Mucor*
Cladosporium	*Mucor*	*Mucor*	
Epicoccum		Yeast	
Bipolaris		Bacteria	
Fusarium			
Cochliobolus			
Dreshslera			
Pyrenophora			

Microbial contaminated grains show unfavourable effects such as discolouration and darkening and an increase in heat due to respiration which can reduce germination causing losses in dry matter (Magan and Aldred, 2007). The sum of these effects results in economic losses in stored grains.

1. 4 Diseases and infections under field conditions.

Like other crops barley often suffers from various diseases affecting all parts of the plant (Table 1.3). Under field conditions, microbes already colonize barley seeds soon after ear emergence from the enveloping leaf-sheaths and can be established in the seed before it germinates. Wind, rain, insects, birds and agricultural practices effectively distribute microbes to initiate infection throughout the growing season. Disease microorganisms may also be transmitted by the recycling of crop residues. In general, barley has different microbial groups related to its growth in different geographic locations. Warm and moist conditions are most likely to encourage fungal growth (Semaskiene *et al,* 2005). Most fungal pathogens are biotrophic but some necrotrophic ones also cause severe losses of yield.

Table 1.3: Fungal diseases attacking barley in the field.

Field barley diseases	Associated fungi
Ramularia leaf spot	*Ramularia collo cygne*
Fusarium head blight	*Fusarium spp*
Scald blotch	*Rhynchosporium secales*
Powdery mildew	*Erysiph graminisf.sp.hordei*
Net blotch	*pyrenophora teres*
Leaf stripe	*Pyrenophora graminea*
Spot blotch	*Cochliobolus sativus*
Rust diseases.	
Leaf rust or brown rust	*Puccinia hordei Otth*
Stem rust	*P. graminis*
Strip rust	*P. stariformis hordei*
Crown rust	*Puccinia coronata* Corda
Smut diseases.	
Covered smut	*Tilletia cariers, and Tilletia foetid*
Loose smut	*Ustilago nuda*
Ergot	*Claviceps purpurea*
Root and foot rot	*Bipolaris sorokinian*

1.4.1 Powdery mildew disease.

Powdery mildew is a common disease in cereals. The disease is important in barley especially in the UK (Wolfe, 1984) and has been cited as the most common plant disease in England and Wales (King, 1977). Mildew causes yield losses and decreases the yield quality which may reach up to 20% in Europe (Czembor, 2002). In barley the disease is caused by the obligate biotrophic fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*).

Blumeria graminis f. sp. *hordei* usually begins rapid growth on the lower leaves and sheaths. Temperatures between 10 and 22°C are favoured for mildew growth. The conidia are produced every seven to ten days and provide repeating cycles of spores. After crop maturity, ascospores in cleistothecia serve as survival structures, but their role in initiating disease is much less important than that of the conidia. After flowering, the disease incidence decreases when the temperatures increase above 25°C. The optimal temperature for conidial production is 20°C and declines rapidly above or below this temperature (Ward and Manners, 1974).

Blumeria graminis f. sp. *hordei* can persist between seasons most likely as ascospores in crop residues and on the soil surface. Ascospores are produced from the cleistothecia and can be splashed or blown onto the leaf surface of emerging seedlings where they germinate and penetrate the cells of the leaf directly. The cleistothecia produced during late summer are resistant to low temperatures, and they allow the fungus to survive in the absence of the host. The asexual life cycle of *Bgh* is more important than the sexual cycle because of the continual production of spores. The infection process starts when wind dispersed conidia on the leaf surface of barley, germinate and immediately begin to produce an extracellular matrix. A short primary germ tube appears within an hour of germination (Kinane *et al*, 2000). Shortly afterwards a second germ tube emerges from the conidia forming an appressorium from its tip. Host penetration by the appressorium is affected by a combination of enzyme activity and mechanical force (Pryce-Jones *et al*, 1999). After penetration, the haustorium develops in the periplasmatic space. Three days after inoculation, the fungal colony is visible to the naked eye. After this point the colony develops conidiophores, which produce a large number of conidia.

The pathogen uses available nutrients from the plant's photosynthesis. These are reduced, thereby decreasing plants growth and vigour, heading and seed filling. Heavily infected leaves and even whole plants can be killed prematurely. Mildew can cause greatest losses when the plants are infected at seedling stage (Both *et al*, 2005). Figure 1.5 illustrates the asexual life cycle of *Bgh*.

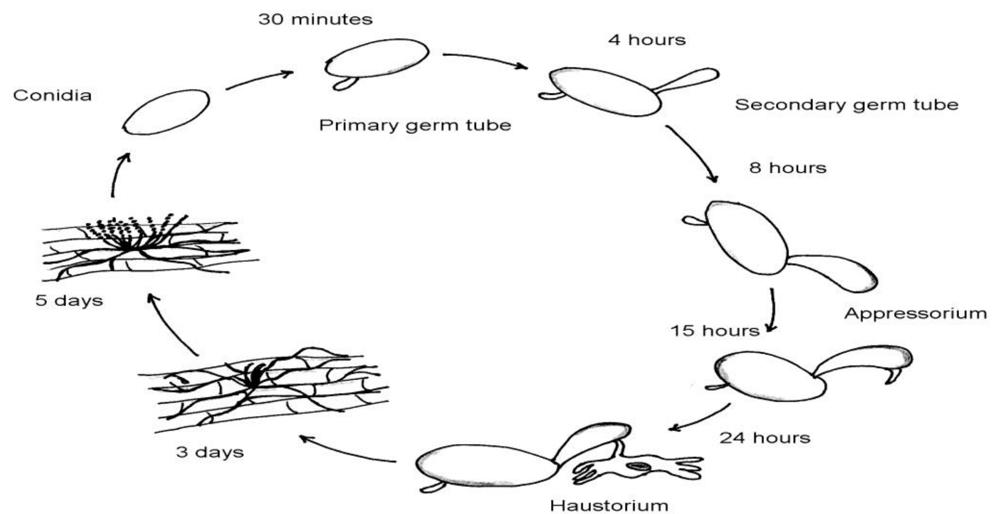


Figure 1.5: Asexual life cycle of *B. graminis f sp hordei*
(drawn after Both *et al*, 2005).

The disease incidence of mildew has increased in recent years due to increased uses of nitrogen fertilizers which resulted in increases in the density of green tissue necessary for pathogen development (Czembor, 2000). Mildew is a cool temperature disease with conidia produced in abundance in cool and moist environments. The highest conidial germination occurs at 12°C with best growth of germ tubes at 21 ^{o}C (Singh *et al*, 2009).

The mildew fungus *Bgh* can overcome basic plant resistance and exploit the host cells by developing feeding structures called haustorium. However, different types of resistance reactions may inhibit the pathogen from invading the host tissue. The most important host reactions appear to be induced after penetration of plant epidermis cells (Gustafsson and Claesson, 1988). The plant response may be to initiate basal defence or to limit fungal growth through compatible interactions (Eichmann and Huckelhoven, 2008). There are several host features that are required for these interactions which may include limiting the factors necessary for

pathogen development, controlling factors used by the pathogen to inactivate or stop host defences or inhibiting host defence responses (Vogel and Somerville, 2000). For example, in wild barley species waxes and other chemical components on the leaf surface may affect conidial germination. Other factors such as humidity on the leaf surface and the age and structure of the leaves also affect germination and growth (Gustafsson and Claesson, 1988). Epicuticular wax features such as chemical composition, crystal structure and hydrophobicity on aerial plant tissues of barley can affect the fungal development before penetration. Epicuticular waxes support conidial adhesion, appressorium formation and secondary hypha growth which promote pathogen development and infection. Studies have shown that the removal of total leaf cuticular waxes lead to a decrease in the proportion of conidial germination (Zabka *et al*, 2008).

A study conducted by Silvar *et al* (2010) revealed that landrace-derived lines from the Mediterranean Coast and Southern regions of Spain have good resistance against powdery mildew and leaf rust diseases. Nevertheless, this resistance is not universal as they are susceptible to virus diseases

The differences between susceptible and resistant spring barley cultivars infected by the pathogen *Bgh* was studied by Kozdoj *et al* (2009) and indicated that there is no difference in shoot length, total number of shoots per plant, number of productive tillers per plant, number of immature tillers per plant, number of dry tillers per plant, ear length and total number of spikelets per ear at the ripening stage. Nevertheless, in infected plants, the grain yield per plant of susceptible barley cultivars is significantly lower compared to resistant barley cultivars.

Field plants of barley can be assessed visually for powdery mildew infection by using visual scoring systems. Alternatively seedling tests in the glasshouse can be used (Jorgensen and Jensen, 1997). Methods other than field screening by using detached leaf infection frequency and biomass per colony assessments using enzyme-linked immunosorbent assay (ELISA) can also be used in the same way as a field screen to select the best phenotypes of barley (Newton and Thomas, 1993).

Polymorphic restriction fragment length polymorphisms (RFLP) markers can be

used to identify the alleles on the *Mla* locus located on chromosome 5 (1H) conferring powdery mildew resistance in barley and to determine genetic differentiation at the DNA level (Schuller *et al*, 1992). For development of future resistance it is important to find how many host genes contribute to the function of resistance genes. It is then necessary to identify appropriate barley cultivars that provide a suitable genetic background to study molecular mechanisms associated with powdery mildew resistance.

A proteogenomics approach has allowed for the identification of many proteins of *B. graminis* f. sp*. hordei* in conidia, hyphae and haustoria. The conidia have been found to contain a few hundred proteins involved in lipid, carbohydrate and phosphate metabolism. For example, enzymes that are required for the breakdown and processing of storage compounds such as lipids and glycogen following germination confirming that conidia are primed for a rapid and effective breakdown of nutrient reserves following germination (Bindschedler *et al*, 2009).

Protection from mildew disease can be achieved using resistant barley cultivars or by spraying foliar fungicides. Quinoxyfen fungicide is used as a protectant fungicide to control mildew disease by interfering with germination and appressorium formation (Wheeler *et al*, 2003). However, as fungicides may leave residues, the selection of varieties with resistance genes is more desirable.

1.4.2 Fusarium head blight disease.

Fusarium head blight (FHB), also called ear blight or scab is a severe disease affecting the maturing grains in cereal crops. More than seventeen different species are implicated in this disease with many species associated with other diseases such as foot rot and seedling blight (Parry *et al*, 1995). The most common fungi causing FHB disease include *Fusarium graminearum*, *F. culmorum, F. poae* and *F. avenaceum*. *Fusarium culmorum* can be found in cooler regions such as north, central and Western Europe including the UK.

The disease causes substantial economic losses to growers as well as reducing seed quality due to grain contamination with mycotoxins which affect human and animal health. FHB has reached epidemic levels in the United States in several years during the last decades, causing yield losses and price reductions related to

decrease seed quality (Windels, 2000). FHB epidemics have been documented in the USA and five Canadian provinces. In barley estimated losses from 1993 to 1999 were up to £ 260 million. Since 1993, North Dakota, South Dakota and Minnesota have lost 73% of their malting barley market with losses in Minnesota alone approaching 95% (Windels, 2000).

Barley quality is also affected by the presence of mycotoxins produced by *Fusarium* infection. The main trichothecene mycotoxins produced by *F. culmorum* are deoxynivalenol, nivalenol (NIV) and zearalenone. Deoxynivalenol (DON) mycotoxins play an important role in disease development and pathogenesis (Wagacha and Muthomi, 2007). Mycotoxins are suggested to be directly involved in enhancing FHB progression by weakening plant cells (Nicholson *et al*, 2004).

The pathogen and activation of host resistance factors for FHB are greatly influenced by environmental conditions. For example, barley resistance response against FHB is influenced by the hydrothermal conditions and the phase of infection. In general, ear infection occurs during anthesis and increases in wet weather or heavy dew as well as in warm temperatures (Osborne and Stein, 2007). In addition nitrogen application increases the levels of *Fusarium* infection in cereal grains considerably (Lemmens *et al*, 2004). The high concentrations of N fertilizer may increase plant water stress, but the effect on FHB is unclear (Geneva: World Health Organization, 2001). Teich (1987) found that the FHB incidence was lower when wheat was fertilized with urea rather than ammonium nitrate suggesting that the form of nitrogen addition is a factor.

Jansen *et al* (2005) found that when *Fusarium* spores germinated on barley and wheat grains, the fungal hyphae move along the epicarp between the lemma and palea reaching the developing grain, where successful infection destroys the grain coat layers and digests the starch and protein accumulating in the endosperm. In general, the host is most susceptible to infection at anthesis and shortly thereafter. Successful infection depends on environmental conditions such as warmth, moisture and heavy dew (Osborne and Stein, 2007). As a protection against infection, the host develops a thicker cell wall in the rachis node. In barley the fungal hyphae are inhibited at the rachis node and rachilla which may limit the infection of adjacent florets. This is in contrast to wheat where the disease

develops laterally more readily. Active resistance to FHB requires expression of genes that control these different paths of infection (Jansen *et al*, 2005).

Barley has two flowering types chasmogamous (open-flowering) and cleistogamous (closed-flowering). The timing of infection differs between these types. For example, cleistogamous cultivars have been shown to be resistant at anthesis but susceptible at 10 days after anthesis (Yoshida *et al* 2007) however, chasmogamous cultivars are susceptible at anthesis. In general, spring barley cultivars are most susceptible to FHB infection after the grain head fully emerges from the leaf sheath but this does depend on environmental conditions and infections may occur up to soft or mid-dough stage (Jordahl *et al*, 2002). All current malting-barley varieties grown in the US are susceptible to FHB and DON accumulation. Resistance genes have been identified in both two-row and six-row barley, but these provide only partial resistance to FHB (Rudd *et al*. 2001) so providing only limited disease control.

Inoculation techniques include single floret inoculation and spray inoculation of the head with a liquid spore suspension (De Villiers, 2009). Single floret inoculation can be done using a pipette or syringe to inject a water suspension of spores into a single central floret at anthesis of the head. Inoculation is usually done with *Fusarium* macroconidia at concentrations of 10^4-10^5 macroconidia ml^{-1} (Gilbert and Woods, 2006). The aqueous solution of macroconidia can be sprayed onto the heads with moisture which facilitate the infection. Irrigation can be used in the field and a mist chamber can be used for glasshouse inoculations. Inoculated heads can be visually scored for disease incidence. In field trials, plants can be sprayed at 50% anthesis and the inoculation is usually repeated one week later to include later developing heads. FHB incidence can be evaluated by scoring the harvested grains at maturity and DON concentration (Rudd *et al*, 2001). Other assessment techniques include1000 grain weight and total grain yield which are the most effective ways of identifying cultivars which are resistant to FHB reducing grain mass (McMullen *et al*, 1997). The percentage of infected grains has been stated to be the best way to identify resistant cultivars when exposed to low infection rates (Parry *et al*, 1995).

Fusarium graminearum persists over winter on infected plant residues on which the fungus overwinters as saprophytic mycelia. Warm moist weather in spring is favourable for the conidia development and perithecia that produce ascospores at the same time as the flowering of cereal crops (Markell and Francl, 2003). The ascospores are windblown or splashed onto the heads from mature perithecia formed on the surface of crop debris (Parry *et al*, 1995). The hyphae develop on the exterior surfaces of florets and glumes, allowing the fungus to grow toward susceptible sites within the inflorescence (Bushnell *et al*, 2003).

The fungus appears to have a brief biotrophic relationship followed by a necrotrophic phase. The necrotrophic stage is associated with an increase in fungal colonization which may lead to plant death (Rubella *et al*, 2004).

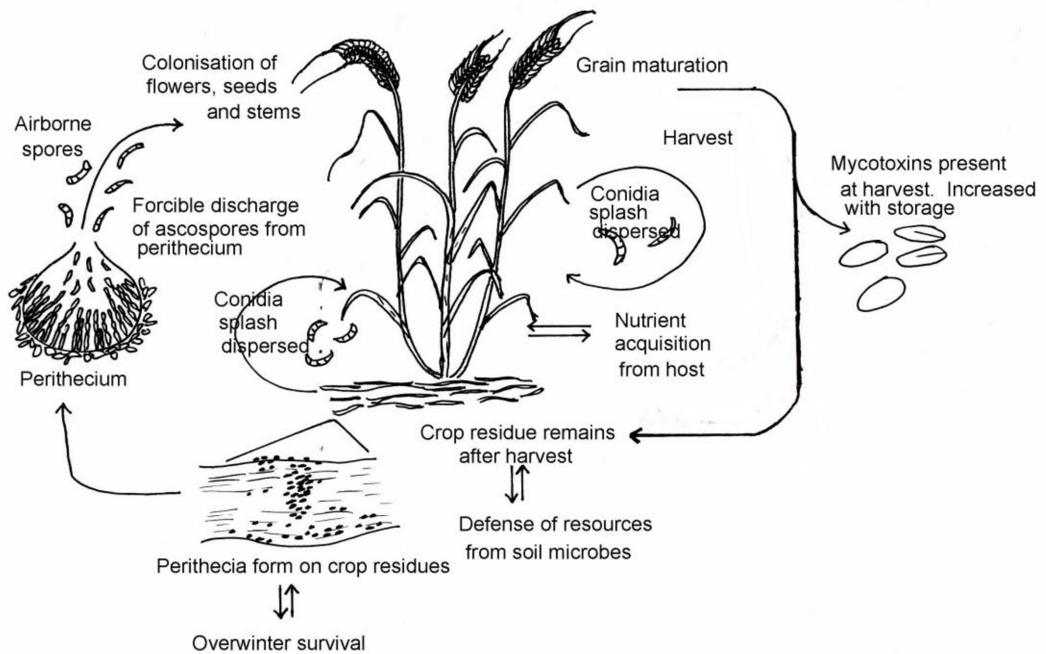


Figure 1.6: Life cycle of *Fusarium graminearum* on barley (drawn after Trail, 2009).

Different agricultural practices in addition to the use of resistant cultivars and fungicides can be used to decrease FHB disease and their mycotoxin contamination. These include choice of cultivar, crop rotation, soil cultivation and fertilizer usage in addition to chemical and biological control (Edwards, 2004). Fungicides available for FHB suppression include Metconazole, Propiconazole,

Prothioconazole, Prothioconazole+Tebuconazole and Tebuconazole (McMullen *et al*, 2008). Biological control methods are regarded as a natural tool to restrict FHB disease and enhance malt quality. Biological control against FHB disease includes bacterial antagonists *Bacillus* AS 43.3 and AS 43.4 against *Gibberella zeae* (Khan *et al*, 2001). *Pseudomonas fluorescens* strains MKB158 and MKB 249 and *P. frederiksbergensis* strain 202 have a high ability to decrease both FHB incidence caused by *F. culmorum* and DON levels on wheat and barley (Khan and Doohan, 2009).

1.5 Resistance to diseases.

Most barley varieties have been developed since domestication. Because of the large repetitive genomes in cereal crops, genomic information is limited and molecular variation among modern varieties is poorly understood (Wicker *et al*, 2009). However, different resistance mechanisms to fungal diseases have been identified in barley including morphological, chemical and localised necrosis (Jorgensen *et al*, 1998; Skadsen and Hohn, 2004; Lewandowski *et al*, 2006).

There are five types of physiological resistance against FHB as reported by Mesterhazy (1995). These are (I) resistance to initial infection, (II) resistance to spread within the head, (III) resistance to grain infection (IV) resistance to yield reduction and (V) degradation or non-accretion of mycotoxins. A genotype must have Type II resistance before Type I resistance can be measured correctly. Assessment of Type II resistance requires point inoculations, where a suspension of conidia is applied to individual florets of the head. Spread of the fungus throughout the head indicates the absence of Type II resistance (Shiner, 2002). Type III resistance to grain infection can be assessed by visual symptoms on infected grains, such as tombstone grains and reduction in grain weight. Type IV resistance can be evaluated by measuring grain yield and Type V resistance can be evaluated by determining DON concentration (Rudd *et al*, 2001).

Barley typically shows Type II resistance to *Fusarium* infection whereby disease does not easily progress from grain to grain in an ear. This suggests that there may be a structural limitation to the fungal penetration. More generally, two-row

barley varieties are cleistogamous showing high resistance against FHB, whereas most six-row varieties are chasmogamous and are either moderately resistant or susceptible to FHB (Yoshida *et al*, 2007).

Stomata and leaf cuticles may also have physical characteristics limiting fungal growth. For example, Niks and Rubiales (2002) showed that the stomatal features of wild barley types *Hordeum chilense* may be hidden under cuticular wax that may prevent rust fungi germ tubes from penetrating the stomata and lead to failure of the pathogen to enter the barley leaf.

Chemical resistance includes the production of inhibitors of fungal growth and of more general toxic agents such as hydrogen peroxide. For example, the cell wall of barley leaves contain thionin polypeptides which are highly toxic to various bacteria and fungi. The toxicity of thionins can be triggered by pathogens and can play an important role in barley defence against pathogen infection (Bohlmann *et al*, 1988).

Disease resistance is known to have genetic determinants. In resistant barley genotype near-isogenic line (NIL) NIL3876-Rdg2a, fungal growth is stopped at the scutellar node of the embryo, whereas in susceptible barley NIL Mirco-rdg2a the fungal development continued to the scutellar node and penetrated the embryo (Haegi *et al*, 2008).

DNA markers for FHB resistance QTLs (Quantitative Trait Locus) have been identified and may be used to speed the introgression of resistance genes into adapted germplasm. This approach can be used to identify and map additional DNA markers linked to genes controlling FHB resistance (Anderson *et al*, 2001). Marker-assisted-selection (MAS) is an important tool to augment current methods to breed for FHB resistance (Nduulu *et al*, 2002). Marker-assisted-selection MAS is a process whereby a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest (i.e. productivity, disease resistance, abiotic stress tolerance and/or quality). This process is used in plant breeding.

Combining genes from multiple sources are required for successful development

of barley cultivars with high levels of FHB resistance. Breeding resistant cultivars could be an effective approach to manage FHB in barley. However, this strategy faces major challenges as all barley genotypes investigated express only partial resistance to FHB (Wingbermuehle *et al*, 2004). Japanese scientists have continued a strong program on FHB resistance in barley (Takeda and Heta, 1989). In Europe, research on FHB resistance has been active in several countries, notably Hungary, Poland, Austria, Germany and the Netherlands (Meidaner, 1997).

A well-studied resistance to fungal disease in barley is the acquisition of the mlo gene which produces localised necrosis in response to hyphal penetration to tissue. This limits fungal growth and so curtails the disease progression. Recessive mlo mutants of the gene are present in some varieties and may account for their susceptibility (Buschges *et al*, 1997).

Examples of historic barley varieties used in this study and their resistance against diseases are listed in Table 1.4 according to the barley pedigree report of Biotechnology and Biological Sciences Research Council (BBSRC, 2002).

Table 1.4: Resistance and susceptibility against diseases in barley.

Cultivar	Resistance genes	Disease resistance	Disease susceptibility
Armelle	*Rh, BRR1* Partial resistance	*Scald/ Rhynchosporium secalis* (Jones and Newton, 2004). Leaf rust/ *Puccinia hordei* (Parlevliet and Ommerenrace**,** 1985).	
Asplund			Powdery mildew disease/ *Erysiphe graminis* (Bjgrnstad, 1986).
Bigo	*PSH-71/* single gene *Rps1. b*	Some of all races of stripe rust/ *Puccinia striiformis* (Chen and Line, 1999). *Puccinia striiformis* f. sp. *hordei* (Chen and Penman, 2005).	
Gloire du velay	Partial primitive Polygenic	Powdery-mildew/ *Erysiphe graminis* (*Newton et al,* 1998)	
Hannchen	*Ruh1*	Barley covered smut/ *Ustilago hordei* (Grewal *et al*, 2008). Net blotch / *Pyrenophora teres* (Jorgensen *et al,* 2000).	*Scald/ Rhynchosporium secalis* (Auriol *et al*, 1978).
Oderbrucker			Stem rust caused by *Puccinia graminis f. sp. tritici* (Brueggeman *et al*, 2002)
Union	*Rph*/ *Rph2*	Leaf rust/ *Puccinia hordei* (Bruckner, 1970)	
Westminster	*BRR-5* and/or *BRR-6*	*Scald/ Rhynchosporium secalis* *Ramularia* (Oxley *et al*, 2006).	

The mechanisms of barley resistance against FHB disease require more understanding. In particular the molecular and physiological bases of the resistance mechanisms are poorly understood. However, most workers have preferred to study the genetic approach to control FHB disease by developing resistant varieties. This approach is a much longer-term strategy compared to that based on the use of fungicides (Dardis and Walsh, 2002) but does require information on the genetic determinants of resistance and of their phenotypic expression.

The possibility of evaluating historic varieties for resistance to contemporary fungal diseases has the potential to contribute to this aim. In barley many DNA-markers for genes can be applied to control diseases. Most of these resistance genes are from landraces or wild species obtained via conventional crossing programs (Manninen and Nissila, 1997). Pickering and Johnston (2005) indicated that the

cultivated barley (*Hordeum vulgare*) has progressed through hybridisation with wild types. For example, the wild species of barley *H. bulbosum* has desirable characters such as disease resistance which would be worth transferring to its cultivated relative.

Breeding from historic varieties with novel disease resistance can support future progress by developing crops which are better adapted to their environment and have more durable disease resistance. However, some changes in the approach to plant breeding may be needed (Cowling, 1996). An assessment of historic varieties for growth characteristics and disease resistance is an initial start towards this aim.

1.6 Barley varieties.

Most modern barley cultivars produce higher yields compared to historic barley cultivars due to the use of fertilizers, herbicides, insecticides and fungicides. However, the high cost of pesticides combined with the low price for barley in addition to the risk to food safety from fungicide residues in grains, have encouraged breeders to scrutinise the inheritance of resistance mechanisms against pathogens. For example, five experiments covering 37 barley varieties were undertaken in Britain between 1880 and 1980 to compare varieties and showed that modern varieties produce higher yield and shorter straw compared to historic barley varieties (Riggsta *et al*, 1981).

Using wild cultivars as a source of novel alleles has produced good success in cereal progress for over 100 years. Progressive gene detection has improved technologies for genetics and breeding associated with better understanding of the factors limiting applied exploitation of exotic germplasm and promises to transform existing and improve new strategies for efficient and directed germplasm utilization. Cytogenetic and molecular analyses are helpful to characterise and produce agronomically valuable recombinant lines achieved from the hybrids.

Many European barley varieties are two-row and spring-sown which produces good quality of malt for malting. However, winter barley produces a greater yield and the best quality of malt characters. To obtain good malt quality and quantity

breeders often use spring barley varieties in the winter barley breeding programs (Rostoks *et al*, 2006). In one early study 55 barley varieties were utilized for crosses for the period of 1928-1937 (Manninen and Nissila, 1997).

Current agriculture and conventional breeding accompanied by the abundant use of pesticides and fertilisers has resulted in the loss of genetic diversity. Progressively landraces are substituted by modern cultivars which are less resistant against abroad range of pests and diseases (Newton *et al,* 2011). A study conducted by Feuillet *et al* (2008) indicated that the gene pool in barley exhibits restricted genetic diversity, which creates concern about the crop ability to overcome harsh environmental conditions and diseases in order to produce greater yield and better quality.

As a result new barley varieties may only last for short periods before they become susceptible to disease or are overtaken by other varieties with greater yield. The selection for short straw varieties in response to mechanised harvesting is one development which may have resulted in loss of associated characteristics.

For malting varieties this progression is tempered with the need for grains with low nitrogen, a high carbohydrate extract and suitable enzymatic digestion. Flavour is also a factor and as a result a number of historic malting varieties such as Halcyon and Marris Otter are still grown in small but increasing quantities. A renewed interest in specialist and traditional food sources suggests that varieties in archive resources would be useful for future consideration. Spring barley varieties recently grown in the UK are listed in Table 1.5.

Table 1.5: Spring barley varieties recently grown in the UK and their resistance and susceptibility against diseases (HGCA 2010-2011).

Malting varieties	Resistance against disease	Susceptibility against diseases
Propino	Mildew	Yellow rust
	Rhynchosporium	Brown rust
Quench	Mildew,	Brown rust
	Rhynchosporium	Yellow rust
		BYDV
Concerto	Mildew	*Rhynchosporium*
	Yellow rust	
	Brown rust	
Forensic	Brown rust	Mildew, *Rhynchosporium*,
	BYDV	Yellow rust
NFC Tipple	Mildew	*Rhynchosporium*
	Brown rust	Yellow rust
	BYDV	
Belgravia	Mildew	BYDV
	Brown rust	
	Rhynchosporium	
	Yellow rust	
Westminster	Mildew	BYDV
	Rhynchosporium	
	Brown rust	
	Yellow rust	
Oxbridge	Mildew	Yellow rust
	Rhynchosporium	BYDV
	Brown rust	
Optic	Brown rust	Mildew, *Rhynchosporium*
	Yellow rust	BYDV

BYDV= Barley Yellow Dwarf Virus

Of the range of modern varieties, Westminster and Tipple have become widely grown in the UK as high yielding barley malting crops. Westminster is a medium-tall variety with good resistance against mildew and *Rhynchosporium* on the HGCA Recommended List 2010. This allows growers to use a low input fungicide programme and it is also has one of the highest untreated yields of 5.6- 6.2 t ha^{-1}. Westminster carries resistance factors *BRR-5* and/or *BRR-6* against *Rhynchosporium* (Jones and Newton, 2004). Trial results carried out at two sites in Scotland in each of four years (2002-2005) revealed that Westminster also had

lower levels of *Ramularia* and had higher green leaf area scores (Oxley *et al*, 2006). The variety Westminster has produced malts with high extract contents (84.1%) and strong activities of proteolytic enzymes (Psota *et al*, 2007) in addition to its high level of germination (Psota *et al*, 2009). This variety has a semi-dwarf gene (*sdw1*) on chromosome 3H and bears mutations in this gene - an important gene with a role in reducing plant height and increasing grain yield. It also has effects on root characters e.g. root length and root weight which enhances the ability to absorb nitrogen and elements from the soil (Ellis *et al*, 2002; White *et al*, 2009).

Tipple is popular high-yielding malting barley in the UK and across Europe. It is shorter than Westminster and has good resistance against mildew and brown rust but it is susceptible to yellow rust, *Rhynchosporium* (HGCA Recommended List, 2010) and *Ramularia* with high scores of green leaf (Oxley *et al*, 2006). Tipple grains have N level of between 1.5- 1.65%. In most situations the recommended amount of N for Tipple barley would be 125-150 Kg/ha (HGCA Recommended List 2010).

In contrast information on historic barley varieties is limited and sporadic in content, particularly where studies were based on barley products such as malt. Information arises from a range of studies with different aims and it is difficult to collate these to make comparisons. As an example details of Chevalier a major historic malting barley in the nineteenth century, provide a useful view of past claims.

1.6.1 Chevalier.

The greatly successful two-row barley cultivar Chevalier was first identified at Debenham, Suffolk in 1819-1820 with distribution to other areas between 1826 and 1827. It became prevalent in Britain in the late nineteenth-century. It is the pedigree of Hallett's which was the favoured barley for many breeders in earlier years (Walton, 1999). Chevalier was the preferred malting barley in England, introduced by Dr. Chevalier during 1830 and was also extensively grown in Scotland (Hunt, 1851).

Chevalier is reported to have many desirable characteristics such as its capability to grow without manure, resistance against drought in addition to having a larger proportion of starch essential for malting purposes.

Chevalier barley was inappropriate to grow on clayey soils because of the weakness of the straw; nevertheless it was reported to grow well on light, inferior and unproductive soils (Milburn, 1843). Historical reports also indicate that it requires a plentiful supply of siliceous substance and benefits from the use of phosphate of lime from crushed bones to improve poor soils (Johnson, 1848). Chevalier is reported to grow very well in dry seasons but is also more liable to lodge in heavy rainy seasons (Milburn, 1842). It is also noted to have a longer flowering period (Hunter, 1952)

Disease scores of Chevalier against net blotch fungus *Pyrenophora teres* indicate its resistance against this pathogen (Jorgensen *et al*, 2000). This feature is worth investigating to determine its diversity and the potential of Chevalier to show resistance is particularly important. Unfortunately, Chevalier became unacceptable for modern agriculture due to its lodging before seed ripening in addition to producing fewer tillers and because its height was difficult to manage with mechanical harvesting.

In summary most modern barley cultivars produce a higher yield compared to historic cultivars due to the use of chemical fertilizers and pesticides in recent years. However, in the past centuries agriculture did not use such fertilizers or pesticides. Although modern varieties may be resistant to specific disease strains, historic barley varieties may have more lasting partial resistance genes. Thus studies on disease resistance in historic barley varieties may help breeding programs to identify major and minor resistance genes. Additionally microscopic investigations may lead to identification of useful features to help understand defence mechanisms.

Some documented research information has been identified on resistance genes in historic barley varieties that could be used in breeding programs to develop modern varieties, but most of this focuses on powdery mildew and rust diseases (Chelkowski *et al*, 2003). There are however no published papers on resistance

genes in historic barley varieties against FHB disease. In spite of the importance of *F. culmorum* on wheat and barley, little published information is available on resistance to this pathogen with most information focussing on *F. graminearum*.

An important question is whether it is possible to identify whether historic barley varieties have resistance genes against diseases? If so can they be incorporated into future breeding trials? Only a few publications have reported on this issue. For example Jones and Davies (1985) examined the level of partial resistance in historic barley varieties against powdery mildew disease at the adult plant stage over three growing seasons. Their results revealed that barley varieties Nottingham, Loibichl, Vellavia, Armelle, Gloire du velay, Chevalier and Union were the most resistant while Plumage, Asplund, Oderbrucker and Dore were the most susceptible to mildew.

A specific question is whether these varieties may have similar resistance to other diseases such as FHB and if so whether there may be similar physiological features associated with this resistance. A relevant hypothesis is that these varieties have similar or better resistance when compared to example contemporary varieties.

1.7 Specific objectives.

The specific objectives to be achieved in this study are:

1. To evaluate example varieties of modern and historic barley for their disease resistance.
2. To determine agronomic characteristics of these barley cultivars.
3. To determine and compare the effect of nitrogen addition on the growth of historic and modern barley varieties.
4. To determine the effect of nitrogen addition on the susceptibility of barley varieties against FHB and powdery mildew diseases.
5. To evaluate grain characteristics of historic barley varieties with the hypothesis that they would produce suitable malt quality compared to example contemporary varieties.

6. To investigate the features that could contribute to resistance mechanisms in barley varieties which could help in breeding programs to develop new FHB resistant varieties combined with greater yield and good quality for malting purposes.
7. To assess malting ability by evaluating the germination rate of modern varieties compared to Chevalier historic variety.
8. To assess the extent of *Fusarium* growth when inoculated onto harvested seeds of modern and historic varieties to determine potential growth during malting.

1.8 Research questions.

1. Do historic barley varieties have resistance mechanisms against FHB disease compared to example contemporary varieties?
2. What are the infection pathways of Fusarium head blight disease in barley?
3. What factors affect resistance features of barley against disease?
4. Can resistant historic barley varieties be incorporated into future breeding trials programs to develop new FHB resistant varieties combined with greater yield and good quality of malt?

Chapter Two

Materials and methods

2.1 Barley samples.

Historic spring barley (*Hordeum vulgare*) varieties (kindly supplied from archive deposits by JIC-Norwich, UK) and the two- row modern barley cultivars (kindly supplied by Nafferton farm at Newcastle University, UK) were used in this study. Their pedigree and year of release are listed in Table 2.1 according to the barley pedigree report (BBSRC, 2002).

Table 2.1: Historic spring barley varieties used in this study and their origin.

Historic barley varieties	Two or six-row	Origin	Pedigree	Year of release
Armelle	2	France	Ceres*Clermont	1974
Asplund	6	Norwegian, northern Sweden	Mixed seed lot selection Swedish land variety	1900-1910
Bigo	6	Netherland	Zeeland land variety selection	1961
Chevalier	2	English landrace	English land race	1820
Dore	6	Swedish	Jamtland variety selection	
Gloire du velay	2	France	Upper Loire barley selection	Before 1957
Hannchen	2	Moravia	Selection (Moravian Hanna selection), Sweden	1893
Loibichl	2			
Nottingham	2	English landrace	English land race	Before 1846
Oderbrucker	6	Manchuria	Manchuria Ex Germany (Wisconsin pedigree 5)	Before 1890
Plumage	2	UK	English variety selection, or Scandinavians barley	1910
Union	2	Germany	(Weihenstephaner 6831*Donaria)*Firlbecks 621, or(Weihenstephaner MR 1*Donaria)*Firlbecks 3	1950
Vellavia	2	France		Before 1957
Modern barley varieties				
Tipple	2	UK	(NFC 497 12Xcork) x Vortex	
Westminster	2	UK	NSL97 - 5547 X Barke	

Materials and methods.

Details of the lineage of some of barley varieties used in this study and their pedigree are listed in Table 2.2.

Table 2.2: Barley varieties used in this study and their pedigree.

Cultivar	Pedigree	Year	Origin
	a- Armelle		
Golf	(Armelle x Lud) x Luke	1983	
Koru	(Armelle x Lud) x Luke	1980	
Claret	(Proctor x HP5466) x Armelle) x Abacus	1979	
Livet	(Dera x Digger) x (TS42/3/5 x Armelle	1998	
(Ellis *et al*, 1997; Russell *et al*, 2000).			
	b- Asplund		
Edda	Asplund x Vega	1945	Sweden
Tammi	Olli x Asplund (Sjakste and Roder, 2004)	1938	Finland
Lise	(Asplund x DS 295) x Varde (Davila *et al*, 1998)		
Fraeg	Asplund x Maskin		
varde	Maskin x Asplund (Manninen and Nissila, 1997; Aastveit and Aastveit, 1984)		
	c- Dore		
Asa	Dore x Vega (Manninen and Nissila, 1997)		
	d- Hannchen		
Seger	Gull x Hannchen (Manninen and Nissila, 1997)		
	e- Union	Year	
Drossel	(FLO-1625/56 X Union) X Ingrid (Schut *et al*, 1997)		
Dukat	CarlsbergII X Union	1971-1976	
Topas	Union X Valticky X Freja	1971-1978	
Favorit	Diamant X Union	1973-1987	
Diabas	(Amsel X Diamant) X (Union X Branisovicky C)	1977-1982	
Koral	[Hana/(Czech) X"{Carlsberg:II X Union) Alsa] X Celechovicky hanacky X 125]	1978-1994	
Fatran	(Sladar X Minerva) X (Sladar X Amsel) X (UnionX Diamant)	1980-1989	
Karat	{(Diamant X (Valticky X B 2145) X (Carlsberg II X Union) X KM-293/70	1981-1989	
Zefir	(Union X Diamant) X (Jantar X Emir)	1981-1988	
Horal	[(Sladar X Minerva) X (Sladar X Amsel) X (Union X Diamant)	1982-1997	
Rubin	[{Valticky X (Algerian X Valticky)} X Union] (Diamant X Nadja)	1982	
(Dreiseit and Jorgensen, 2000).			

2.2 Fungal isolates and culture media.

The fungal isolate of powdery mildew used in this study, *Blumeria graminis* f. sp. *hordei* (*Bgh*), was obtained from infected barley plants in nearby fields at Nafferton experimental Farm, Newcastle, UK and used directly to infect barley plants.

The fungal isolate *Fusarium culmorum* strain *Fu42* used in this study was kindly supplied by Dr. Paul Nicholson, JIC, Norwich. Stock cultures were maintained on malt agar at 4 ^{o}C after growth at 25 ^{o}C. To obtain spores, barley seeds were soaked in water for 24 hours and autoclaved in a 250ml conical flask. The fungus *F. culmorum Fu 42* was grown on this sterilized barley for one month at room temperature. Grains were then shaken in sterile distilled water and filtered through four layers of muslin as spore suspension and maintained at -20 ^{o}C. The fungal conidia were counted and adjusted to obtain a spore suspension of $5x10^5$ spores' ml^{-1}.

2.3 Planting and growth conditions for glasshouse experiments.

In 2009 barley cultivars were sown under glasshouse conditions at Close house-Newcastle under natural daylight conditions. The temperature ranged between 8 and 28°C and the photoperiod was approximately 15-18 hours. Seeds of each variety were sown in John Innes No 3 compost in 2 litre pots, 225 mm diameter and 235 mm tall.

In 2010, barley cultivars were grown under glasshouse conditions at the University of Sunderland. Plants were grown in a randomized array under natural spring and summer daylight conditions. The photoperiod was approximately 15-18 hours and the temperature ranged from 9 to 33°C.

Two groups of experiments were conducted; the first group was conducted in compost. Seeds for each variety were sown in John Innes No 3 compost in 100 mm diameter, 150-mm tall pots to score symptoms of FHB and mildew diseases. The second group was conducted in sand with three experiments [nitrogen experiment 1 (N1), nitrogen experiment 2 (N2) and nitrogen experiment 3 (N3)].

Plants were watered with different levels of nitrogen. The design of all experiments with different barley cultivars and different levels of nitrogen was arranged as a randomized complete block.

2.4 Disease assessments.

2.4.1 Powdery mildew disease.

All barley cultivars grown under field and glasshouse conditions in 2009 and 2010 were scored for mildew infection. The percentage leaf area covered with mildew was recorded according to methods developed by the Genres project CT98-104 (Genres, 1999).

Mildew infection was scored by using the following scale: 0 = immune no visible symptoms; 1 = necrotic, areas with few mycelium; 50 = large pustules with some chlorosis, necrosis and substantial sporulation, and 70% = large pustules, no necrosis and abundant sporulation. The disease rating from 0 to 1% was considered resistant; ratings between 50 and 70% were classified as susceptible.

2.4.2 FHB disease.

Heads for all barley cultivars grown under field and glasshouse conditions in 2009 and 2010 were scored for FHB infection. Barley heads were visually assessed for FHB infection to determine the percentage of infected plants and heads (as a measure of disease incidence) and of grains (as a measure of disease severity). The percentage of *Fusarium* damaged grains was determined based on grain colour and degree of shrivelling (tombstone grains) for each head.

2.5 Preparation technique for inoculations and spraying methods.

2.5.1 Powdery mildew disease.

The leaves of all barley cultivars were artificially inoculated with the isolate of *Blumeria graminis* at a rate of one infected plant for each ten plants. Seven-day-old plants whose first leaves were fully expanded were inoculated with a *Bgh* pathogen by shaking the conidia from infected donor plants onto the leaves of

barley varieties. Plants from all cultivars were scored for mildew infection after one week. Scoring for the second set of control plants not artificially infected was conducted on three different occasions.

2.5.2 FHB disease.

Once barley heads had started to form on the earliest variety, they were sprayed with the spore suspension of $5x10^5$ spores' ml^{-1} containing 4 drops of tween 80 as detailed in 2.2 until the water started to run off. Since the varieties flowered at different times, barley cultivars were sprayed with the spore suspension twice per week repeated seven times over a three weeks period. The control treatments were sprayed with sterile distilled water containing four drops of tween 80.

The above method was used to inoculate plants grown under field and under glasshouse conditions for both locations, Sunderland and at the JIC-Norwich.

2.6 Culture media and microbiological analysis.

Microbiological media Potato dextrose Agar (PDA) obtained from Oxoid Ltd'' was prepared according to manufacturer's instructions by dissolving the ingredients in distilled water. The media was autoclaved for 20 min at 121°C, cooled to approximately 50°C and dispensed into petri dishes.

Investigations of microbes which had been isolated from barley seeds on PDA for six days at 25^oC were also undertaken. Five seeds were placed in each 9cm Petri-dish with six replicates for each variety. Three petri-dishes were cultured with seeds directly and a further three cultured after surface sterilization with 3.5% (w/v) sodium hypochlorite.

After sterilizing, the grain samples were washed three times in sterile distilled water before culturing on PDA media. The seeds from both groups (without and with surface sterilization) were placed on solidified agars using sterile forceps.

2.7 Nitrogen content in barley leaves and seeds.

To determine the levels of nitrogen stored in stem bases and leaves (Lewis *et al*, 1982), barley leaves and seeds from 2009 and 2010 plants grown under field and glasshouse conditions were prepared for CHN analysis.

Dry leaves and seeds were ground separately and samples (20-50mg) of each variety were subjected to CHN analysis by Chemispec at the University of Sunderland. For this analysis all the Carbon, Hydrogen and Nitrogen contained in the sample are converted into CO_2, H_2O and N_2 respectively and combusted in oxygen at a temperature in excess of 180^oC. These gases are then quantified using high precision thermal conductivity detectors. The percentage of carbon, hydrogen and nitrogen was obtained by measuring the responses of S-benzyl thiuronium chloride standards compared to the response of the samples.

2.8 Harvest and post-harvest techniques.

All barley cultivars were harvested in September after measuring plant height and tiller number. Barley heads were separated and grain weight for each barley cultivar was determined. All plant roots were removed and barley leaves and stems were dried at 60^oC to constant weight. Nitrogen content in seeds was also determined for both field and glasshouse experiments as detailed in 2.7.

Chapter Three

Effect of powdery mildew and Fusarium head blight diseases on growth and yield of historic and modern barley cultivars

3.1 Introduction.

3.1.1 Powdery mildew disease in barley.

Barley is susceptible to a range of diseases as noted in Chapter One. Observations on plants in preliminary screening trials reported here indicated that mildew and Fusarium head blight were particularly evident in some varieties and could be assessed in more detail.

Powdery mildew is a common disease affecting a wide range of plants. The disease can be found on the leaf surface and appears as white fluffy patches, which turn grey when they mature. The spots are of different sizes and can cover the leaf completely and sometimes symptoms appear on the heads. Leaves turn yellow-brown as the disease progresses. Most of the mildew life cycle is characterised by a long mainly haploid phase with a short diploid phase. Sexual reproduction includes the formation of cleistothecia and ascospores and asexual reproduction involves the formation of conidiophores that produce haploid spores, called conidia as detailed in 1.4.1. The disease pathogens are obligate biotrophic parasites, obtaining nutrients from their host via an intracellular feeding structure known as haustoria. Ridout (2009) indicated that most mildew species have a high degree of host specialization by infecting only the one or a few closely related host plants and a number have gene-for-gene resistance interactions with their host plant. A study conducted by Eichmann and Huckelhoven (2008) revealed that the mildew fungus is able to affect basic host resistance in addition to employing host cells to form a haustorium in epidermal cells. However, these plant factors may be regulating basal defence adversely or may sustain fungal development.

In barley, mildew infection is caused by the obligate biotrophic fungus (*Blumeria graminis* f. sp. *hordei* (*Bgh*) formerly known as *Erysiphe graminis* f. sp. *hordei*). Shen (2004) indicated that the mildew pathogen *Bgh* can infect the epidermal cell layer of leaves and aerial parts of the plant. As a result, the development of fungal hyphae growth on the leaf surface leads to the appearance of mildew symptoms. He also indicated that the best temperature for the development of mildew infection is 20°C. Last (1955) observed that mildew infection increases between the end of May and mid-July and is associated with active growth of barley.

Sreeramulu (1964) found that the number of conidia in the air is influenced by the rain which can reduce their number to a very low level.

The disease kills leaves by decreasing photosynthetic activity which leads to reduction in yields especially as affected areas increase. The infection can also affect plant growth, for example by reducing shoot number and hence yield (Last, 1962a). Increasing levels of mildew disease is associated with yield reductions in infested barley possibly due to the reduction in tiller and grain number and in grain size (Scott and Griffiths, 1980).

Schulze-Lefert and Vogel (2000) showed that various pathways could govern resistance against mildew fungus. Some of these pathways are involved in determining isolate-specific fungus responses, while other pathways enhance broad-spectrum defence responses such as host-cell death and rapid cell-wall restructuring.

In general, barley varieties carrying the mlo allele of the Mlo locus are resistant against all mildew pathogen isolates. The mlo-11 allele from Ethiopian landraces currently controls mildew resistance in cultivated European spring barley elite varieties (Piffanelli *et al*, 2004). In barley varieties with the mlo allele, infection is followed by rapid development of subcellular cell wall appositions and papilla leading to blocked fungal penetration in these appositions. The antifungal compound p-coumaroyl-hydroxyagmatine is also found to increase (von Ropenack *et al*, 1998).

Overall, powdery mildew disease becomes more important in dry and warm areas (Czembor, 2000). Most modern spring barley cultivars such as Propino, Quench, Concerto, NFC Tipple, Belgravia and Westminster have the mlo gene which provides resistance against powdery mildew disease (HGCA, 2010). However, mlo genes make barley susceptible to the fungal pathogen *Magnaporthe grisea* which causes blast disease (Jarosch *et al*, 1999).

3.1.2 Fusarium Head Blight disease.

Fusarium Head Blight (FHB) disease, also known as ear blight or scab, is a serious disease of wheat and barley, and can also infect other cereal hosts including maize, oat, rice and rye (Osborne and Stein, 2007). *Fusarium spp.* which have been isolated from FHB in Europe are; *F. graminearum*, *F. culmorum* and *F. avenaceum.* Other species are *F. poae, F. cerealis, F. equiseti, F. sporotrichioides, F. tricinctum, F. acuminatum, F. subglutinans, F. solani, F. oxysporum, F. verticillioides, F. semitectum* and *F. proliferatum* which are less pathogenic (Bottalico and Perrone, 2002). *Fusarium culmorum* is typically found in cold regions such as the UK, Northern Europe and Canada (Desjardin, 2006). *Fusarium graminearum* is normally found in Canada, United States, North America, China and other portions of Asia (Bai and Shaner, 1994; Hatcher *et al*, 2003; Goswami and Kistler, 2004; Gale *et al*, 2002).

In Europe, FHB can also be caused by *Microdochium nivale* and *M majus* pathogens (formerly *M. nivale* var*. nivale* and *M. Nivale* var*. majus* (Xu *et al*, 2007) in addition to *Fusarium* species. This pathogen is particularly found in cool and wet conditions. However, *Microdochium spp.* do not produce mycotoxins (Nicholson *et al*, 2003; Xu *et al*, 2007). Growth of *Fusarium* species depends on specific environmental conditions, especially temperature and humidity (Nicholson *et al*, 2004). For example, *F. culmorum* inoculation causes greater disease symptoms at 20°C than at 16 °C, while *F. graminearum* causes greater disease symptoms at 16°C than at 20°C. However, both cause higher yield losses at 20°C (Brennan *et al*, 2005). In general, optimal growth temperatures are between 20-25°C for *F. culmorum* and 25°C for *F. graminearum* (Doohan *et al*, 2003; Brennan *et al*, 2005).

Epidemics of FHB disease caused economic losses in the UK estimated at more than £620 million in wheat and barley due to lower yields, shrivelled “tombstone” grains and reductions in market grade of grain due to DON mycotoxins reducing grain quality (Ali *et al*, 2007). In the United States, economic losses from FHB have been estimated in wheat and barley production together over £1.50 billion for the period from 1993 to 2001 (Nganje *et al,* 2004).

Different approaches can be used to reduce FHB disease particularly by planting resistant varieties, using fungicide treatment at heading and crop rotations. In wheat, genetic resistance to FHB is generally expressed as a quantitative trait, presumptively related to many minor genes and a few major genes that confer resistance (Osborne and Stein, 2007). However, few studies have been conducted for barley. Recently Jia *et al* (2010) showed that resistance against FHB is partial and inherited. The main FHB resistant Quantitative trait locus (QTL) on barley was found on chromosome 2H Bin 8 and 2H Bin 10 while another QTL able to decrease DON mycotoxin accumulation was identified on chromosome 3H (QTLs is a region of a chromosome containing genes that are believed to make a significant contribution to the expression of a complex phenotypic trait). The identification of new sources of resistance will provide a valuable resource for controlling FHB in barley which is the aim of this study.

Studies of resistance to FHB disease in two-row barley showed inherent Type II resistance (Langevin *et al*, 2004; Foroud and Eudes, 2009), whereby disease progression between spikelets was limited. This resistance to disease spread is also found in oats, possibly due to the large spacing between the florets. However, this contrasts with wheat which has Type I resistance, characterised by spreading between the spikelets. The production of mycotoxins was also suggested to be a factor in the spread of disease by aiding pathogens to overcome Type II resistance (Langevin *et al*, 2004). A possible locus controlling spike type has been suggested as contributing to Type II resistance in barley (Ma *et al*, 2000).

Fusarium culmorum and *F. graminearum* fungi attack the developing barley head during the flowering period through grain growth and thus effects grain size, weight, protein content and the baking quality of flour (Del Ponte *et al*, 2007; Wang *et al*, 2005). The fungus penetrates the grain through the brush hairs and progresses slowly along the pericarp and cross-cells (Skadsen and Hohn, 2004) eventually reaching the starchy endosperm and damaging the grain structure (Jackowiak *et al*, 2005). The fungus also stimulates the production of catechin which affects haze formation of malt products (Wettstein *et al*, 1980) and is another deleterious effect of the disease.

Environmental conditions are the most critical factor for FHB disease compared to tillage practices and fertilizer applications (Lori *et al*, 2009). The moisture content of ears, warmth, humidity and rainfall are highly favourable for FHB disease development and mycotoxin production. Mycotoxins are water-soluble and translocate between tissues or are leached from source tissues. Continuation of post-flowering moisture has important effects on enhancing FHB disease by increasing damaged grains and DON mycotoxins (Cowger *et al*, 2009). Semaskiene *et al* (2005) found that the development of *Fusarium* species in spring organic grain was greater than in winter grain. Nitrogen levels also affect the extent of FHB disease and mycotoxin levels (Lemmens *et al,* 2004; Eggert *et al,* 2010).

Deoxynivalenol (DON) has multiple effects on plant growth through inhibiting protein synthesis, inhibiting cell division and inhibiting cell wall thickening as a defence reaction. All these effects lead to loss of chloroplast pigments associated with bleaching. At early stages of infection, DON was found to delay senescence and in later stages caused bleaching by degradation of chloroplasts and other components of cells and cell death (Bushnell *et al*, 2010). The detection of DON is an important diagnostic in assessing the quality of cereal grains as well as indicating the presence of disease. Polymerase chain reaction (PCR) analysis and Enzyme-linked immunosorbent assay (ELISA) tests play an important role in the assessment of disease by detecting DON itself or genes for the production of DON (Leisova *et al*, 2006). Specific analysis and identification requires chromatography and mass spectroscopy.

There is often no correlation between FHB visual symptoms and DON mycotoxin content. FHB can be asymptomatic where DON is detectable and vice versa (Hill *et al*, 2006). The factors which determine *Fusarium* growth and DON levels depend upon ecological conditions (Champeil *et al*, 2004) and on grain characteristics (Liu *et al*, 1997). Environmental conditions that encourage *Fusarium* pathogens to produce trichothecene mycotoxins are moisture during and after flowering (Edwards, 2007). A high correlation has also been reported between DON in barley and DON in malt and between DON in malt and wort colour (Schwarz and Horsley, 2006).

Fusarium species are able to produce a number of mycotoxins including the trichothecenes and enniatins that contaminate infected grains. DON is the most widespread and important mycotoxin produces by *Fusarium spp* (Nicholson *et al,* 2004). Fusariotoxins are the most common mycotoxins in cereals such as wheat, barley and oats (Tekauz *et al*, 2008).

There are four types of trichothecenes (A-D) depending on the central molecular structure and the number of associated hydroxyl and acetoxy groups (Sokolovic *et al*, 2008). The most common trichothecenes are Type A (such as HT-2 toxin, Diacetoxyscirpenol and T-2 toxins) and Type B (such as Nivalenol, Deoxynivalenol and Fusarenon) (Champeil *et al*, 2004).

Molecular methods based upon PCR allow the detection of species which are capable of producing mycotoxins (Nicholson *et al,* 2004). A positive correlation was reported between *Fusarium* DNA and DON levels in barley by using real-time PCR assays which have been usefully applied to barley for FHB assessment with symptomic and asymptomic grain (Demeke *et al*, 2010). A new method to detect FHB infection even with asymptomatic heads is by using *Fusarium* protein equivalent (FPE) with the AUDPC (area under the disease progress curve). It is a practical alternative to AUDPC and DON content for use in research breeding programmes (Slikova *et al*, 2009). As measure of *Fusarium* biomass, FPE can be determined with a double antibody sandwich (DAS) link ELISA (DAS-ELISA) by using Fusarium-specific antibodies and protein standards (Wolfarth *et al*, 2011). DAS is a test for antigens using an application of the ELISA method in which material being tested is added to wells coated with known antibody. The presence of antigen fixed to the antibody coat can be determined either directly, by adding antibody linked to the enzyme of the indicator system or indirectly, by first adding unlabelled known antibody, the attachment of which to the antigen can be demonstrated by addition of immunoglobulin-specific antibody linked to the enzyme.

A large number of PCR assays are available for the detection of several genes which are involved in trichothecene and enniatin biosynthesis and to detect species that are capable of producing the associated mycotoxins (Nicholson *et al*, 2004). For example, numerous PCR assays have been developed for the *Tri5*

gene that encodes trichothecene synthesis. This gene is present in *Fusarium* species that are capable of producing trichothecene (Edwards *et al*, 2001). *Tri13* and *Tri7* genes from the trichothecene biosynthetic group are responsible for converting DON to NIV (*Tri13*) and are also responsible of acetylation of NIV to 4-acetyl nivalenol (*Tri7*) (Chandler *et al*, 2003).

ELISA methods are able to detect asymptomatic disease infested samples with low FHB but high DON levels. Moreover, ELISA analysis for *Fusarium* antigens is a practical alternative method to quantify DON. Because of its speed, it is particularly valuable to plant breeders interested in monitoring FHB (Hill *et al*, 2008) and to processors of the grain as it can be applied on site using portable instruments.

Chromatographic methods of DON determination can be performed with gas chromatography (GC) with either electron capture or mass spectrometry (MS) (Mirocha *et al*, 1998). GC-MS or electronic nose detection is able to predict DON by using volatile compounds (Pentane, methylpyrazine, 3-pentanone, 3-octene-2-ol and isooctylacetate) (Olsson *et al*, 2002). High-performance liquid chromatography (HPLC) combined with mass spectrometric (HPLC/MS) for the DON detection and DON derivatives is quick, sensitive and overcomes several problems such as inability to obtain straight calibration curves, memory effects, matrix interferences and matrix response enhancement (Berger et *al*, 1999). However, HPLC methods with ultraviolet (UV) are applicable only to B trichothecenes and require very effective clean-up procedures. Immunoaffinity columns for clean-up combined with HPLC-FLD (fluorescenic detection) have been shown to give the best detection of DON-derivative (Klotzel *et al*, 2005).

Fusarium infection is not limited to field barley but may also progress during storage and malting of grains. During the malting process Laitila *et al* (2002) reported that the levels of *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. oxysporum* increased after the steeping stage, while the levels of *Fusarium spp.* decreased at kilning stage.

In this study by Laitila *et al* (2002) *Fusarium* counts were determined by placing 100 randomly selected barley grains on a wet filter paper. Germination of grains

was prevented by wetting the filter paper with 15-20ml 2%-2.4-D-sodium salt solution (2.4-dichlorophenoxy acetic acid) and incubated at 25°C for 21d. *Fusarium* species were identified under a stereomicroscope on the basis of typical colony form and colours. Identification was confirmed by conidia morphology with a light microscope. The determinations of fungi were expressed as the per cent *Fusarium*-contaminated barley grains in the total number of grains (Laitila *et al*, 2002).

Some DON mycotoxins introduced in infected barley may be lost during steeping as the grains are washed. However, *Fusarium* is able to grow and produce mycotoxins during steeping, germination and kilning (Wolf-Hall and Schwarz, 2002) and these will be transferred into finished malt products with deleterious results. For example, as well as contaminating the malt high levels of T-2 toxin (>1000 ng/g) can inhibit α-amylase and β-amylase activity so affecting further processing.

Another consequence of *Fusarium* growth on grains is gushing of malt beverages. Gushing is defined as unprompted over-foaming which occurs when a packaged malt product is opened and is often attributed to a heavy *Fusarium* infection of barley or malt (Schwarz *et al*, 1996). Heavy infection with *F. poae, F. graminearum* and *F. culmorum* create the gushing tendency of malt beverages. It also increases enzyme activities in malt which generates a darker wort colour, along with increased soluble nitrogen and higher free amino nitrogen content (Sarlin *et al*, 2005a). Gushing in malt products occurs when the concentration of hydrophobins produced by *Fusarium* increases above 250 µg g-1 in malt (Sarlin *et al*, 2005b).

3.1.3 Objectives.

The Objectives of the current study reported here were to compare barley cultivars for their susceptibility to mildew and FHB diseases and for agronomic characteristics. Included in the study were both modern, elite varieties from the HGCA recommended list and historic varieties that may provide greater genetic diversity for resistance sources.

The characteristics of barley varieties differ in both growth features and seed yield

and have implications for crop management and suitability for final use, particularly for specialist applications such as malting.

Plant height, tiller number and overall plant biomass are important characteristics which may contribute to grain yield and which have both genetic and environmental determinants.

3.2 Methods and experimental procedures.

3.2.1 Field experiments.

3.2.1.1 Preliminary field trials, 2009 (F 2009).

In 2009, fifteen historic and modern spring barley varieties as listed in Table 2.1 were grown in a small-scale trial for initial assessment under field conditions. These experiments were conducted at Nafferton farm, University of Newcastle between 28th of April and 15th of September in three plots of 1x1 m^2 in manured soil previously seeded with wheat. A rate of 20 seeds from each variety was planted randomly in each plot at a final density of 300 seeds m^2 to agree with standard planting recommendations (HGCA). Growth was uncontrolled for disease with no pesticide application.

These experiments were conducted to determine the growth characteristics of different barley cultivars and to score for diseases occurring naturally under control (uninfected) conditions. All barley varieties were scored for mildew and FHB diseases developing naturally. The percentage of infected area, leaves and plants with mildew were scored during barley growth before harvest. The percentage of infected heads and plants with FHB were taken before and at final harvest when all varieties had reached GS 92.

The characteristics of different barley varieties including plant height, number of tillers, dry weight and grain yield were taken at harvest as detailed in 2.8. Nitrogen content in seeds was determined as detailed in 2.7.

3.2.1.2 Large scale field trial, 2010 (F 2010).

In the second year, 2010 at JIC-Norwich, six barley cultivars, Armelle, Chevalier, Oderbrucker, Tipple, Vellavia and Westminster were grown for scoring of FHB disease under control (uninfected) and infected conditions between 15th of March and 1st of September. Five replicate plots of 1 m^2 for each infected and control treatment were planted with 8 grams of seeds of each variety.

Fungicide treatment was not applied to the crop, standard husbandry was used throughout.

3.2.2 Glasshouse trials.

3.2.2.1 Glasshouse trial, 2009 (G 2009).

Further experiments with the same fifteen barley varieties mentioned above in 3.2.1.1 were conducted on 29th April under glasshouse conditions at Close House nurseries in Newcastle. All barley varieties were scored for powdery mildew disease under both uninfected conditions as control and under artificial infected conditions in an adjacent glasshouse under the same conditions as detailed in 2.5.1.

Ten seeds of each variety were sown in John Innes No 3 compost in pots with six replicates for each variety (three for mildew infection and three for control treatment). Plants were thinned to six plants per pot. The design of all experiments with different barley cultivars was arranged as randomized complete block.

3.2.2.2 Glasshouse trial, 2010 (G 2010).

In 2010, seven barley cultivars, Armelle, Chevalier, Oderbrucker, Plumage, Tipple, Vellavia and Westminster were grown in compost between 20th April and 2nd of September at the University of Sunderland under glasshouse conditions.

Forty pots containing a litre of John Innes No 3 compost for each barley cultivar were planted with three seeds (thinned to one plant after germination) per pot under FHB infected conditions as described in 2.5.2. The same seven barley

varieties were grown in a separate glasshouse without infection and with twenty pots for each variety to act as controls. The design of all experiments with different barley cultivars was arranged as randomized complete block.

3.2.3 Scoring methods for powdery mildew and FHB diseases in barley.

3.2.3.1 Powdery mildew disease incidence under field and glasshouse conditions.

All barley varieties grown under field and glasshouse conditions were scored for mildew disease infection in inoculated and un-inoculated (natural infection) plants. The levels of mildew infection on all barley varieties (percentage of leaf area covered with mildew, percentage of infected leaves and percentage of infected plants) were determined four times during the barley growth season at seedling and adult stages as detailed in 2.4.1.

3.2.3.2 Fusarium Head Blight (FHB) disease assessment.

Heads of barley cultivars with FHB symptoms on uninfected plants (F 2009) and on infected plants grown under field and glasshouse conditions in 2010 (F 2010, G 2010) as detailed in 3.2.1 and 3.2.2, were scored for FHB infection as detailed in 2.4.2 at 1-2 weeks intervals three times during the growing season and one more time at harvest. Varieties grown without infection as controls under glasshouse conditions in 2010 (G 2010) were scored for FHB infection just once at harvest.

3.2.4 Polymerase chain reaction (PCR) preparation.

The *Fusarium culmorum* species specific primers C51 (forward, 5’-AAC TGA ATT GAT CGC AAG C-3’) and C51 (reverse, 5’-CCC TTC TTA CGC CAA TCT C-3’), enzymes and other chemicals for PCR were obtained from Sigma Co and were the highest purity available for relevant studies.

3.2.4.1 Real Time-PCR protocol.

DNA extraction was conducted at JIC in Norwich using the following protocol. DNA was extracted from 2g of milled grain samples using the CTAB method and

quantified using a Nano Drop spectrophotometer (Brandfass and Karlovsky, 2008). Grain samples were diluted to a standard 10ng μl^{-1} concentration before use and purified *Fusarium* DNA diluted to make a standard curve (0.004, 0.04, 0.4, 4.0, and 40 ng/ul). Quantification of *F. culmorum Fu 42* DNA was determined using the standard curve and amounts of DNA expressed as pg fungus DNA per ng total DNA (Nicholson *et al*, 1998).

PCR was conducted using the primers specified above and performed using Bio-Rad CFX 96 and Bio-Rad 1000 thermo cyclers. The composition of PCR master mix was 12.5µl SYBR green jumpstart (Sigma Aldrich), F and R primers 10ul, H_20 3ul and DNA 5 ul (10ng/ul). PCR conditions were: 95°C for 10 min followed by 39 cycles of 94°C for 10 s and 62.9^{o}C for 30 s. Melt curves were conducted between 65.0^{o}C and 95.0^{o}C at 0.5^{o}C increments.

3.2.5 Mycotoxin analysis.

Two methods were used to analyse DON mycotoxins in barley grains; High-performance liquid chromatography-mass spectrometric (HPLC-MS) and Rapid One Step Assay (ROSA).

3.2.5.1 HPLC-MS method.

All barley samples were analysed for mycotoxins at JIC in 2010 using the following procedure. Fifty grams of ground barley was weighed into a glass cortex tube and polyethylene glycol 8000 and reverse osmosis water added. Each tube was vortexed for 2 minutes and sonicated for 30–40 minutes. Samples were then centrifuged at 7°C and 10,000 rpm in a Sorvall SS34 centrifuge rotor. The supernatant produced was removed to a clean Eppendorf tube and centrifuged for 2 minutes at top speed in a bench centrifuge. The supernatant was then removed to a fresh tube and stored at -20°C.

Before analysis 1 ml of the sample was taken and added to DON extraction columns and eluted with 1 ml of 100% methanol. Samples were evaporated to dryness and reconstituted in 300µl 10% acetonitrile and finally filtered using Vectaspin tubes. 50µl of samples and 30µl of DON standard was injected for analysis.

3.2.5.2 Rapid One Step Assay (ROSA) procedure.

Twenty grams of ground sample was placed in a clean extraction container and five times the weight of deionised water added. The samples were shaken for 2 minutes and left to settle. 1ml was taken from the top of this solution and centrifuged for 10 seconds. 100 µl of this was added to 1.0 ml DON dilution buffer, mixed well and 300 µl added to a ROSA-M test strip. After 10 minutes incubation the strip was read on the ROSA-M reader. The sensitivity of the reader is 0 to 5000ppb DON and values below 1000 were judged to be acceptable.

3.2.6 Barley growth characteristics.

All barley cultivars grown under field conditions, 2009 (F 2009) and under glasshouse conditions, 2010 (G 2010) were harvested in September after measuring plant height and tiller number. Grain weight and plant dry weight were determined as detailed in 2.8. Nitrogen content in seeds was also taken post-harvest for field experiments, 2009 (F 2009) as described in 2.7.

3.2.7 Statistical analysis.

All data taken as a percentage of infection of area, leaves, plants, heads or grains were assessed for differences between group mean values using one-way and two-way ANOVA. All data sets were checked for normality of distribution before analysis. Equality of variances was checked using Levenes tests. Where raw data was not normally distributed or the Levenes test is significant (< .05) the data was transformed to log values before analysis. Other ways were also used to transform the data including angular, Logit, square root, cube root, to the power $(x)^{1/4}$, to the power $(x)^{1/8}$, to the power $(x)^{1/3}$, reciprocal (1/x), cosine and sine. When transformations were unsuccessful one-way non-parametric ANOVA (Kruskal Wallis) tests were used to test if there is a significant difference between groups. However, this test can only provide information about overall significance, not between group variations.

Evaluating the relationship between powdery mildew incidence and varieties in the prediction of the grain yield, tiller number, plant height and dry weight or between

mildew and varieties in the prediction of FHB incidence were determined by analysis of covariance which was carried out on all data by using ANCOVA within SPSS.

3.3 Results.

3.3.1 Powdery mildew disease.

3.3.1.1 Field trial, 2009 (F 2009).

Powdery mildew was observed to be the major visible disease other than FHB in the field. Natural infection of barley cultivars with *Blumeria graminis* f. sp. *hordei* in 2009 resulted in mildew disease symptoms that were visible on the leaf surface of barley plants (Figure 3.1). Analysis of symptoms has shown that mildew levels at the seedling stage of barley were more prevalent compared to the later stages (Table 3.1). In the first assessment at GS 38-39, statistical analysis revealed significant differences between varieties (F (14, 33) = 5.918, $P < .001$). Loibichl, Gloire due velay, Armelle, Bigo and Chevalier exhibiting high levels of mildew with over 40% of infected area. In the third assessment (GS 58-59), statistical analysis also revealed significant differences between varieties (F (14, 33) = 2.255, P = .027). Oderbrucker, Dore, Chevalier, Gloire due velay and Bigo showing significantly higher levels of mildew infection compared to modern and other historic varieties.

Figure 3.1: Mildew disease symptoms in Chevalier barley.

Table 3.1: Percentage of leaf area covered with mildew and percentage of infected plants in historic and modern barley varieties in the field, 2009 (F 2009).

GS 38-39			GS 58-59			
Variety	(2 or 6 row)	% of infected area (all leaves)	Variety	(2 or 6 row)	% of infected area (upper leaves)	% of infected plants
Tipple	**(2)**	**0**	**Tipple**	**(2)**	**0**	0
Westminster	**(2)**	**0.17**	Union	(2)	0	0
Plumage	(2)	7.64	**Westminster**	**(2)**	**0**	0
Vellavia	(2)	18.18	Armelle	(2)	3	1.66
Oderbrucker	(6)	21.85	Hannchen	(2)	3	1.66
Hannchen	(2)	29.89	Nottingham	(2)	3	1.66
Nottingham	(2)	33.83	Vellavia	(2)	6.66	5
Asplund	(6)	35.81	Asplund	(6)	7.40	15
Dore	(6)	38.63	Loibichl	(2)	11.50	3.33
Union	(2)	39.65	Oderbrucker	(6)	13.60	8.33
Loibichl	(2)	40.60	Dore	(6)	16.67	5
G d Velay	(2)	41.96	Chevalier	(2)	18.33	5
Armelle	(2)	44.20	G d velay	(2)	18.33	5
Bigo	(6)	47.22	Bigo	(6)	20.37	13.33
Chevalier	(2)	47.67	Plumage	(2)	30	1.66

GS 38-39 One-way parametric ANOVA					GS 58-59 One-way parametric ANOVA				
P	df	F	LSD	CV	P	df	F	LSD	CV
< .001	14	5.918	6.265	.62	.027	14	2.255	5.767	.96

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference and CV coefficient of variation

The level of mildew infection varied considerably over all varieties, in the first assessment no or low infection levels were evident in modern varieties Tipple and Westminster and in historic variety Plumage. Conversely, in other varieties the levels of mildew infection ranged from 21.85% in Oderbrucker to 47.67% in Chevalier (Table 3.1). Further assessments were made from the upper leaves of the adult plants and indicated that there were low levels or no symptoms in most varieties at later stages of growth (GS 61-69) under field conditions, however higher levels of mildew infection were evident on old leaves compared to young leaves on the same stem.

Because of the possibility that the different varieties were at different growth stages during this assessment, a definitive comparison is not possible. More

definitive comparisons were thus conducted in 2009 and 2010 under glasshouse conditions.

3.3.1.2 Mildew disease incidence under glasshouse conditions, 2009 (G 2009).

Results of mildew incidence from glasshouse experiments in 2009 showed heavy mildew infection levels as detailed in Table 3.2. Nevertheless, the results from all treatments indicated that modern varieties (Tipple and Westminster) and historic variety Plumage always showed lower levels of mildew infection at all growth stages compared to other historic barley varieties.

Statistical analysis for the first disease assessment (GS 39-45) revealed significant differences between varieties ($F\,(12, 25) = 3.959$ and 3.695, $P = .002$ and .003) for the percentage of infected area and leaves respectively. Chevalier barley had significantly higher mildew incidence, however modern varieties had no or low mildew levels compared to historic barley varieties.

Statistical analysis for the second disease assessment (GS 51-65) revealed significant differences between varieties ($P = .001$). The percentage of infected area was higher than 40% overall historic varieties except Plumage which had just 26%.

Statistical analysis for the percentage of infected leaves at this assessment revealed significant differences between varieties ($F\,(14, 30) = 21.077$, $P < .001$). The varieties Loibichl, Chevalier, Hannchen, Nottingham, Bigo and Gloire du velay had significantly higher mildew incidence above 58% compared to modern varieties and Plumage historic barley variety (2.18- 13.31%).

In general, the results obtained from the glasshouse experiments gave approximately similar results to those obtained from field experiments particularly from the first disease assessment. Nevertheless, in July and August 2009 this disease became less prevalent under field conditions even in the susceptible varieties mentioned above.

Under glasshouse conditions, the disease became heavier over time and the heavy mildew infection contributed to most barley varieties succumbing to heavy aphid infection.

The early termination of this experiment indicated that the plants were under stress and that the results may not be reliable for an exhaustive comparison. However, observations as the disease progressed suggested that modern varieties of barley were particularly resistant to mildew and that Chevalier was particularly susceptible.

Table 3.2: Percentage of leaf area covered with mildew (from all leaves) and the percentage of infected leaves in mildew treatment under glasshouse conditions, 2009.

(GS 39-45)						(GS 51-65)		
Variety (2 or 6row)		% infected area	Variety (2 or 6row)		% of infected leaves	Variety (2 or 6row)	% infected area	percentage of infected leaves
Tipple	**(2)**	**0**	**Tipple**	**(2)**	**0**	**Westminster (2)**	**0**	**2.18**
Westminster	**(2)**	**0**	**Westminster**	**(2)**	**0**	**Tipple (2)**	**1**	**4.22**
Union	(2)	0.83	Union	(2)	3.61	Plumage (2)	2	13.31
Nottingham	(2)	2.27	Vellavia	(2)	6.90	Union (2)	4	21.51
Vellavia	(2)	2.64	Oderbrucker	(6)	6.99	Armelle (2)	4	33.55
Loibichl	(2)	3.18	Plumage	(2)	7.06	Asplund (6)	4	35.56
Oderbrucker	(6)	3.30	Nottingham	(2)	7.45	Vellavia (2)	5	36.14
Dore	(6)	3.60	G d velay	(2)	10.03	Dore (6)	5	43.32
Armelle	(2)	3.70	Asplund	(6)	10.07	Oderbrucker (6)	5	48.83
G d velay	(2)	5.81	Armelle	(2)	13.97	Loibichl (2)	5	58.32
Hannchen	(2)	6.40	Bigo	(6)	17.79	Chevalier (2)	5	59.54
Asplund	(6)	7.20	Loibichl	(2)	20.62	Hannchen (2)	5	64.93
Bigo	(6)	7.50	Hannchen	(2)	21.50	Nottingham (2)	5	65.08
Plumage	(2)	8.40	Chevalier	(2)	31.46	Bigo (6)	5	68.80
Chevalier	(2)	37.08	Dore	(6)	32.54	G d velay (2)	6	84.28

% infected area (GS 39-45) One-way parametric ANOVA (Log)					% infected leaves (GS 39-45) One-way parametric ANOVA (Log)					% infected area (GS 51-65) One-way non-parametric ANOVA				% infected leaves (GS 51-65) One-way parametric ANOVA				
P	df	F	LSD	CV	*P*	df	F	LSD	CV	*P*	df	Chi-square	CV	*P*	df	F	LSD	CV
.002	12	3.959	2.380	1.52	.003	12	3.695	4.598	.92	. 001	14	36.051	.41	< .001	14	21.077	5.788	.60

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference and CV coefficient of variation

3.3.1.3 Mildew disease incidence under glasshouse conditions, 2010 (G 2010).

The results of mildew disease incidence for seven barley varieties grown under glasshouse conditions for FHB assessment as described in 3.2.2.2 are shown in Table 3.3. The results showed that the percentage of mildew infection was higher in Chevalier barley compared to modern barley cultivars. On Chevalier, the percentage of infected area, leaves and plants with mildew infection was 42.81, 61.16 and 100% respectively at GS 51. The final assessment at GS 77 from the upper leaves suggested that Chevalier, Vellavia and Oderbrucker were more susceptible to mildew compared to modern barley cultivars (Table 3.3).

Statistical analysis at GS 38 revealed no significant differences between barley varieties (Armelle, Chevalier and Vellavia) ($P = .197$) for the percentage of infected area, however there were significant differences between these varieties for the percentage of infected leaves ($F(2, 41) = 7.534$, $P = .002$). Statistical analysis at GS 45 for historic barley varieties revealed significant differences between barley varieties ($F(4, 82) = 53.281$and $F(4, 82) = 24.894$, $P < .001$) for the percentage of infected area and leaves respectively. Statistical analysis at GS 51 for barley varieties without Westminster variety revealed significant differences between barley varieties for the percentage of infected area ($F(5, 181) = 92.697$, $P < .001$) and also for the percentage of infected leaves ($P < .001$). The final assessment at GS 77 from the upper leaves revealed significant differences between Chevalier, Oderbrucker and Vellavia for the percentage of infected area ($F(2, 47) = 10.607$, $P < .001$) (Table 3.3).

Overall, the results from both growing seasons (F 2009) and (G 2010) indicated that the natural mildew infection is more prevalent in Chevalier, Vellavia, Dore, Oderbrucker, Asplund and other historic barley varieties with 100% of Chevalier plants showing incidence under glasshouse conditions. However, modern barley cultivars Tipple and Westminster in addition to Plumage historic barley showed resistance against mildew disease with very limited infection.

Table 3.3: Percentage of leaf area covered with mildew, percentage of infected leaves and percentage of infected plants under glasshouse conditions, 2010 (G 2010).

% of infected area Variety (2 or 6 row)		From all leaves GS 38	GS 45	GS 51	(upper leaves) GS 77
Armelle	(2)	1	1.78	7.39	0
Chevalier	(2)	4.71	35.79	42.81	46.83
Oderbrucker	(6)	0	4.44	12.24	15.4
Plumage	(2)	0	2	3.21	0
Tipple	**(2)**	**0**	**5.59**	**8.40**	**0**
Vellavia	(2)	3.45	4.80	8.05	33.33
Westminster	**(2)**	**0**	**0**	**0**	**0**
% of infected leaves		**GS 38**	**GS 45**		**GS 51**
Armelle	(2)	13.89	9.32		30.96
Chevalier	(2)	21.57	57.94		61.16
Oderbrucker	(6)	0	25.41		26.17
Plumage	(2)	0	8.10		18.41
Tipple	**(2)**	**0**	**13.25**		**24.06**
Vellavia	(2)	7.66	13.00		16.98
Westminster	**(2)**	**0**	**0**		**0**
% of infected plants		**GS 38**	**GS 45**		**GS 51**
Armelle	(2)	8	6		55.36
Chevalier	(2)	41.33	100		100
Oderbrucker	(6)	0	47.83		57.14
Plumage	(2)	0	48.31		59.26
Tipple	**(2)**	**0**	**4**		**45.45**
Vellavia	(2)	22	72.5		82.5
Westminster	**(2)**	**0**	**0**		**0**

Percentage of infected area					Percentage of infected leaves				
One-way parametric ANOVA (Between Armelle, Chevalier and Vellavia) GS 38									
P	df	F	LSD	CV	P	df	F	LSD	CV
. 197	2	1.689		1.15	.002	2	7.534	13.737	1.03
		(Angular)		(Between historic barley varieties) GS 45					
< .001	4	53.281	8.832	.091	< .001	4	24.894	18.119	.73
		(Logit)		(Between all varieties (Westminster excluded) GS 51					
< .001	5	92.697	7.119	1.15	One-way non-parametric				
					P	df	Chi-square		CV
					< .001	6	170.445		.87
(Between Chevalier, Oderbrucker and Vellavia) GS 77									
< .001	2	10.607	29.724	1.72					

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference and CV coefficient of variation

3.3.2 Fusarium Head Blight (FHB) resistance among barley varieties.

3.3.2.1 Small-scale trial for initial assessment (F 2009).

An initial screen of fifteen barley varieties for FHB disease which occurred naturally under field conditions at Nafferton farm in 2009 was conducted as a preliminary test for choosing varieties in the field trial at JIC. The results indicated that resistance potentially occurred in the historic variety, Chevalier (Figure 3.2).

A- Tipple (tombstone grains)

B- Westminster (tombstone grains)

C- Oderbrucker (Six-row barley)

D- Chevalier

Figure 3.2: Effect of FHB disease on different barley varieties; A-C susceptible varieties and D- Chevalier resistance barley variety.

Disease rating for FHB differed among barley cultivars tested from the field experiment in 2009. The percentage of infected plants in the field ranged between 0-11.6 before harvest at GS (61-89) (Table A4). At harvest (GS 92), the percentage of infected plants ranged between 13.7 and 61.2% while the percentage of infected heads varied between 4.4 and 30.6% indicating a wide range amongst the varieties (Table 3.4).

The results indicate that low disease levels occurred in Chevalier barley cultivar. Chevalier showed a lower percentage of infected heads compared to other barley cultivars.

At harvest, statistical analysis indicated that the percentage of infection differed significantly between varieties (F (14, 30) = 3.176, P =. 004 and F (14. 30) = 2.155, P = .038) for the percentage of infected plants and heads respectively. Chevalier had significantly lower FHB incidence compared to other historic and modern barley cultivars (Table 3.4). Interestingly, the six-row varieties had a slightly lower level of plant infection than the two-row varieties but a higher level of head infection.

Mildew was prevalent on plants at early stages of growth. To know whether powdery mildew disease at the seedling stage affects the percentage of FHB infection levels, a preliminary analysis to evaluate the validity of an ANCOVA test indicated that the interaction between the incidence of mildew and varieties in the prediction of the percentage of infected heads is not significant (F (14, 15) = .416, p = .946). It was thus appropriate to proceed to the ANCOVA analysis.

ANCOVA analysis indicated that the relationship between the covariate (mildew) and the dependent variable (FHB infection) is not significant (F (1, 29) = .542, p = .468) indicating that there is no significant effect of mildew disease on FHB incidence. ANCOVA results combined with a poor relationship between the percentages of infected heads with FHB and the percentage of infected area with mildew for barley cultivars at harvest (R^2= 0.001, P= .868) (Figure 3.3), indicated that mildew had little effect on FHB disease while barley varieties had more influence on the percentage of FHB infected heads. The percentage of FHB

infected heads among the seven barley varieties ranged between 4.4 in Chevalier to 30.6 in Dore.

Table 3.4: Percentage of infected plants and heads with FHB under field conditions at harvest, September, 2009.

Variety (2 or 6 row)		Percentage of infected plants	Variety (2 or 6 row)		Percentage of infected heads
Chevalier	(2)	13.69	Chevalier	(2)	4.41
Plumage	(2)	27.77	Union	(2)	14.11
Bigo	(6)	28.04	Hannchen	(2)	15.53
Loibichl	(2)	32.85	**Westminster**	**(2)**	**15.60**
Oderbrucker	(6)	34.13	Loibichl	(2)	16.54
Dore	(6)	34.66	G d velay	(2)	17.38
Union	(2)	36.57	Plumage	(2)	18.70
Hannchen	(2)	38.52	Nottingham	(2)	22.11
Asplund	(6)	38.70	Vellavia	(2)	22.73
G d velay	(2)	40.76	Armelle	(2)	22.74
Armelle	(2)	42.83	**Tipple**	**(2)**	**24.37**
Nottingham	(2)	43.94	Bigo	(6)	24.51
Tipple	**(2)**	**44.63**	Oderbrucker	(6)	27.62
Westminster	**(2)**	**51.97**	Asplund	(6)	29.82
Vellavia	(2)	61.20	Dore	(6)	30.58

Percentage of infected plants One-way parametric ANOVA					Percentage of infected heads One-way parametric ANOVA				
P	df	F	LSD	CV	*P*	df	F	LSD	CV
. 004	14	3.176	6.624	.37	.038	14	2.155	5. 020	.46

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference and CV coefficient of variation

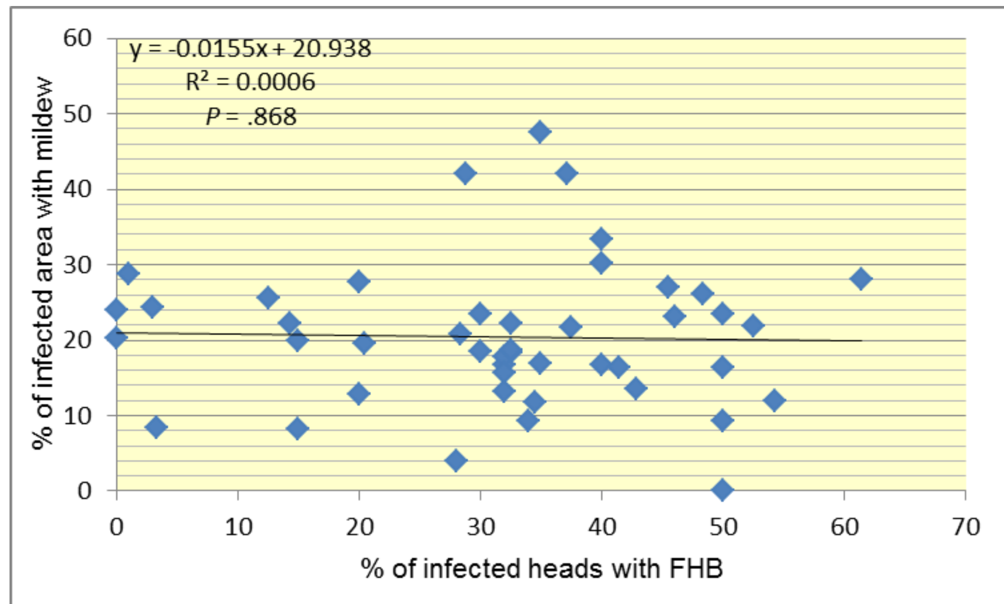


Figure 3.3: Regression between the percentages of infected heads with FHB and the percentage of infected area with mildew for barley cultivars at harvest, field experiment, 2009 (F 2009)

3.3.2.2 Glasshouse trial for FHB assessment, 2010 (G 2010).

The repeat glasshouse screen in Sunderland in 2010 gave similar results, and further confirmed resistance of Chevalier and Plumage varieties against FHB infection. The percentage of infected grains and heads with FHB disease increased sharply with time in most varieties particularly in Vellavia and Tipple compared to Chevalier which increased slightly. The percentage of infected grains increased from 0, 1.41 and 4.42% at the first assessment (GS 61-65) (Table A5) to 5.73, 52.84 and 58.61% at harvest (GS 92) for Chevalier, Tipple and Vellavia cultivars respectively (Table 3.5). Similarly, the percentage of infected heads increased from 0, 10.91 and 25% (Table A5) to 30.92, 92.09 and 94.28 for Chevalier, Tipple and Vellavia cultivars respectively (Table 3.5). Overall, Chevalier showed a lower percentage of infected grains and heads during the growing season and at harvest (Figure 3.4). FHB disease also occurred naturally on plants grown under control conditions on uninfected plants but at low levels.

At harvest statistical analysis revealed significant differences between varieties (F (6, 63) = 25.338 and 12.128, P < .001 for the percentage of infected heads on infected plants and percentage of infected grains on control plants respectively)

and also between infected and uninfected plants (F (1, 183) = 30.180, P < .001 for the percentage of infected grains).

To know whether the level of powdery mildew disease appearance on most historic barley varieties affects FHB infection, a preliminary analysis to evaluate the validity of an ANCOVA test indicated that the interaction between the incidence of mildew and varieties in the prediction of the percentage of FHB infected grains is not significant (F (5, 57) = .691, p = .632). It was thus appropriate to proceed to the ANCOVA analysis. ANCOVA analysis indicated that the relationship between the covariate (mildew) and the dependent variable (FHB infection) is not significant (F (1, 62) = .150, p = .700). This shows that there is no relationship (effect) between the mildew disease and the FHB variable. ANCOVA results indicated that mildew disease had little effect on the percentage of infected grains while barley varieties had more influence on the percentage of infected grains. The percentage of FHB infected grains for the seven barley varieties ranged between 5.73 in Chevalier to 58.61 in Vellavia (Table 3.5).

Table 3.5: Percentage of grain infection and head infection with *F. culmorum* at harvest for barley cultivars grown under control and infected conditions, glasshouse experiment, Sunderland, 2010 (G 2010).

	a- Grain infection						
	Varieties (2 or 6 row)						
Treatment	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	**Tipple (2)**	Vellavia (2)	**Westminster (2)**
Control	5.40	3.70	15.24	5.47	**18.23**	28.13	**21.65**
Infected	29.25	5.73	20.33	14.53	**52.84**	58.61	**22.90**
	b- Head infection						
	Armelle	Chevalier	Oderbrucker	Plumage	**Tipple**	Vellavia	**Westminster**
Control	27.5	19	55.50	43.33	**71.83**	71.67	**41.51**
Infected	74.91	30.92	61.28	57.33	**92.09**	94.28	**69.49**

		% Grain infection							% Head infection			
		One-way parametric ANOVA (Angular)							One-way non-parametric ANOVA			
		P	df	F	LSD	CV			P	df	Chi-square	CV
Control	V	< .001	6	12.128	7.958	.84	Control	V	< .001	6	27.198	.51
		One-way non-parametric ANOVA							One-way parametric ANOVA (Angular)			
		P	df	Chi-square					P	df	F	LSD
Infected	V	< .001	6	54.836			Infected	V	< .001	6	25.338	11.788
	V	< .001	6	64.455					One-way non-parametric ANOVA			
		One-way parametric ANOVA (Angular)							P	df	Chi-square	
		P	df	F	LSD			V	< .001	6	58.069	
	T	<.001	1	30.180	27.222			T	< .001	1	16.812	

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference, CV coefficient of variation, V varieties and T FHB treatment

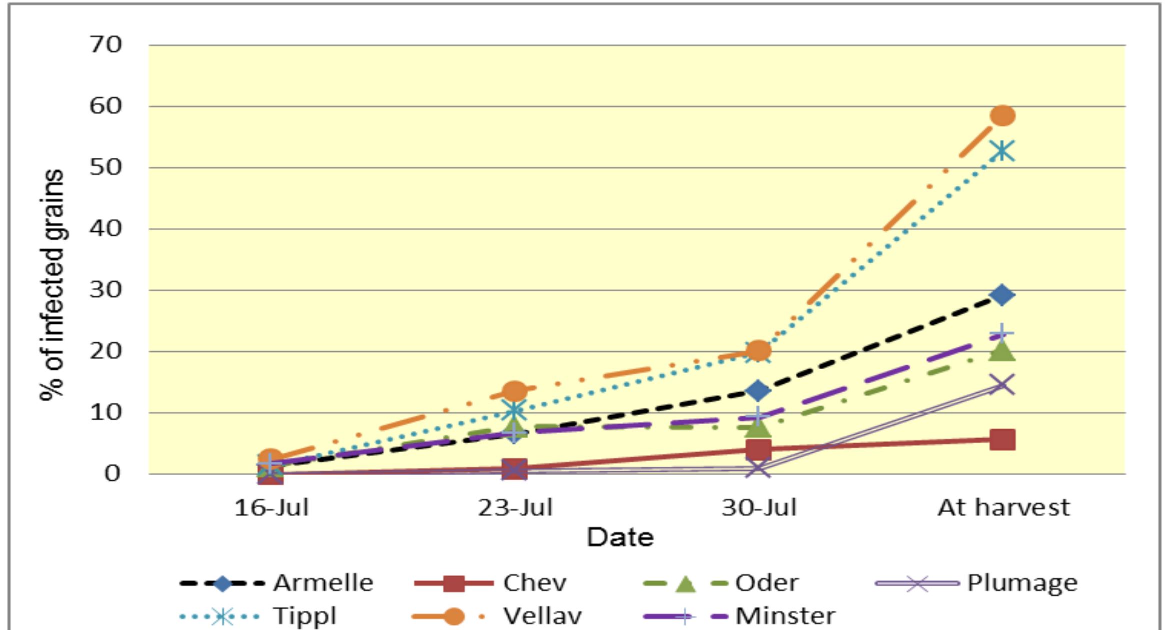


Figure 3.4: Percentage of infected grains with FHB during growing season until harvest for infected barley varieties under glasshouse conditions, Sunderland, 2010.

The relationship between the percentages of infected heads and plants from field experiment results in 2009 is significant (R^2= 0.260, P < .001). The relationship between the percentage of infected grains and heads from glasshouse trial in 2010 is also significant (R^2= 0.700, P < .001) (Figures 3.5-3.6). Moreover, a comparison of correlation between the scoring values for the first, second, third and harvested head and grain infection levels in glasshouse plants indicated no trend or decline in association (0.97, 0.92, 0.89, and 0.94 respectively). This would suggest that the infection recorded was a result of individual grain infection, consistent with Type I infection, and not from lateral transfer from grain to grain.

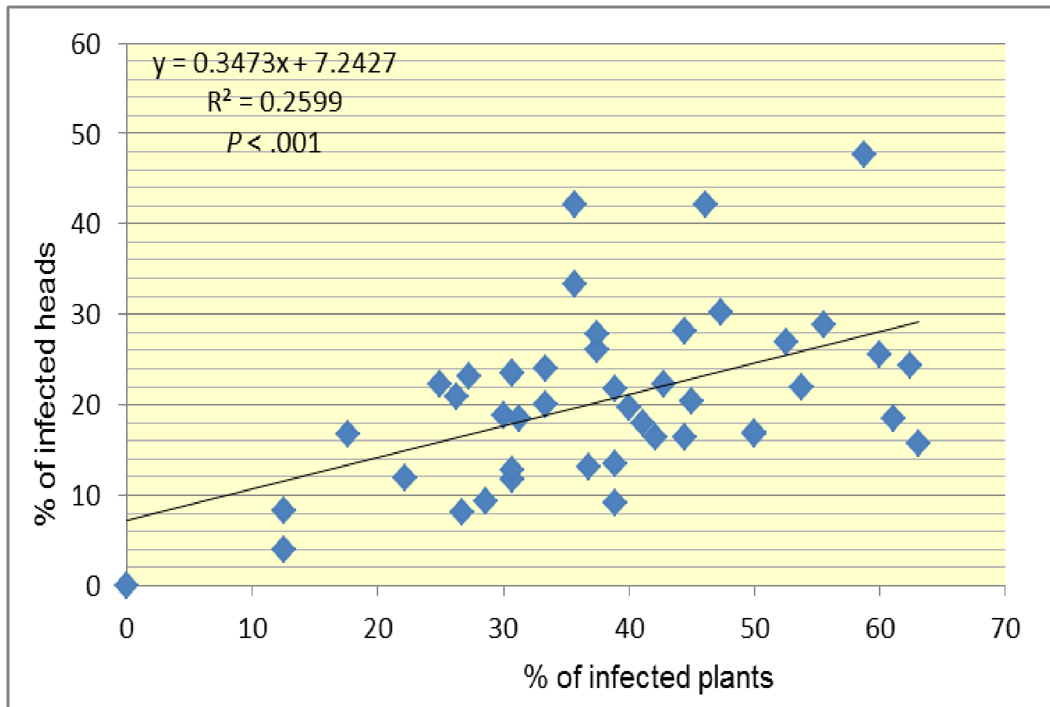


Figure 3.5: Regression between the percentages of infected heads and plants for barley cultivars at harvest, field experiment (2009).

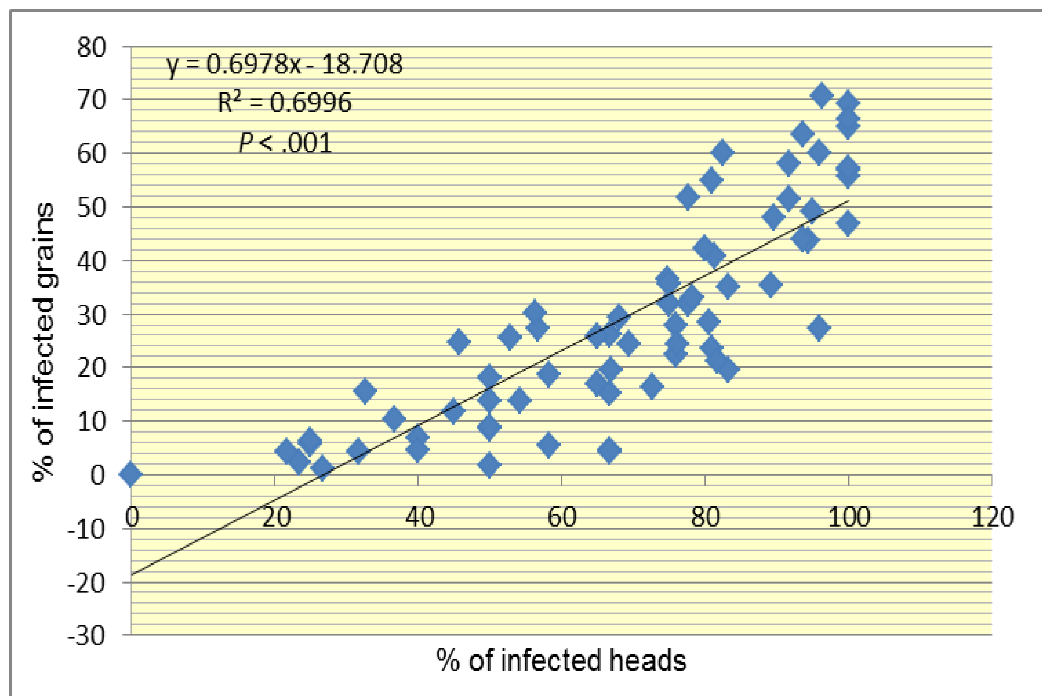


Figure 3.6: Regression between the percentages of infected grains and heads for barley varieties at harvest under glasshouse conditions (2010).

3.3.2.3 Large scale field trial (F 2010).

Based on the results from the 2009 field trial, six varieties were selected for comprehensive evaluation in a field trial at JIC in 2010. The results from JIC of barley at GS (87-89) suggested that resistance to FHB was evident in Chevalier and Armelle whereas Tipple, Vellavia and Westminster varieties were susceptible (Table 3.6). The range of disease incidence in this experiment was between 1.40-25.3% and 16.0 - 86.4% for infected grains and heads respectively.

Statistical analysis revealed significant differences between varieties (F (5, 24) = 18.906, P < .001 for the percentage of infected grains) (Table 3.6). In general, modern varieties Tipple and Westminster and Vellavia historic variety showed a greater infection levels compared to Armelle, Chevalier and Oderbrucker.

Table 3.6: Percentage of infected grains and heads with *Fusarium culmorum* under field conditions at JIC, 2010.

	Variety (2 or 6 row)					
	Armelle (2)	Chevalier (2)	Oderbrucker (6)	**Tipple (2)**	Vellavia (2)	**Westminster (2)**
% infected grains	6.03	1.40	10.18	**25.32**	16.20	**15.52**
% infected heads	52.80	16	57.73	**86.4**	79.2	**76.8**

% Grain infection					% Head infection			
One-way parametric ANOVA (Angular)					One-way non-parametric ANOVA			
P	df	F	LSD	CV	*P*	df	Chi-square	CV
< .001	5	18.906	4.050	.69	. 001	5	21.187	.44

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference and CV coefficient of variation

Overall, the results for both years 2009 and 2010 and under both field and glasshouse conditions showed higher disease levels in modern varieties (Tipple and Westminster) and other historic varieties such as Asplund, Dore, Vellavia and Oderbrucker. In contrast, low levels of infected plants, heads and grains at most dates of assessment and at harvest in Chevalier barley indicated that this variety has strong resistance against *F. culmorum* infection.

A summary of the comparison between infection levels in harvested plants from the various trials is shown in Table 3.7.

Table 3.7: Summary of infection levels in historic and modern varieties over field and glasshouse trials 2009 - 2010.

		Percentage of infected plants			
Varieties (2 or 6 row)		Field 2009 (Control)	Sunderland 2010	Sunderland 2010	JIC 2010
Armelle	(2)	42.83			
Chevalier	(2)	13.69			
Plumage	(2)	27.80			
Vellavia	(2)	61.20			
Tipple	**(2)**	**44.63**			
Westminster	**(2)**	**51.97**			
Oderbrucker	(6)	34.13			
		Percentage of infected heads			
Varieties		Field 2009 (Control)	Sunderland 2010 (Infected)	Sunderland 2010 (control)	JIC 2010 (Infected)
Armelle	(2)	22.74	74.90	27.50	52.80
Chevalier	(2)	4.41	30.90	19.00	16.00
Plumage	(2)	18.70	57.33	43.40	
Vellavia	(2)	22.73	94.28	71.70	79.20
Tipple	**(2)**	**24.37**	**92.10**	**71.80**	**56.40**
Westminster	**(2)**	**15.60**	**69.50**	**41.50**	**76.80**
Oderbrucker	(6)	27.60	61.30	55.50	57.70
		Percentage of infected grains			
Varieties		Field 2009	Sunderland 2010 (Infected)	Sunderland 2010 (control)	JIC 2010 (Infected)
Armelle	(2)		29.30	5.40	6.00
Chevalier	(2)		5.70	3.70	1.40
Plumage	(2)		14.50	5.50	
Vellavia	(2)		52.80	28.10	16.20
Tipple	**(2)**		**52.80**	**18.20**	**25.20**
Westminster	**(2)**		**22.90**	**21.70**	**15.50**
Oderbrucker	(6)		20.30	15.20	10.20

3.3.3 Effect of flowering date on FHB infection level.

Observation in this study on the flowering date revealed differences between barley cultivars. For example, Oderbrucker was the first variety to produce heads, Vellavia and Tipple barley cultivars also produced heads earlier than other varieties and were susceptible to FHB disease. However, Chevalier which had resistance against FHB produced heads later. A listing of flowering date of barley cultivars during the 2009 season's growth is shown in Table 3.8.

Table 3.8: Summary of flowering dates of barley varieties.

Varieties	Type/ No. rowed	Flowering date
Armelle	2	21-6/ late
Asplund	6	15-6/early
Bigo	6	25-6/late
Chevalier	2	24-6/late
Dore	6	15-6/early
Gloire du Velay	2	25-6/late
Hannchen	2	23-6/late
Loibichl	2	16-6/early
Nottingham	2	24-6/late
Oderbrucker	6	13-6/first one
Plumage	2	19-6/middle
Tipple	**2**	**14-6/early**
Union	2	15-6/early
Vellavia	2	15-6/early
Westminster	**2**	**16-6/early**

Statistical analysis revealed no significant correlation between the percentage of infected grains and flowering date (-.592, P = .081). However, the correlation between the percentage of infected heads and flowering date is significant (-.672*, p = .049).

3.3.4 Quantification of *Fusarium* DNA.

Analysis of grain DNA by real time PCR from 2010 field samples revealed a lower amount of fungal DNA in Chevalier and Armelle varieties in contrast with other barley varieties (Table 3.9).

Table 3.9: Analysis of *F. culmorum* by using C51 primers to determine the relative amount of *Fusarium* DNA in the total DNA extracted.

Variety (2 or 6 row)		pg per ng DNA
Armelle	(2)	0.68
Chevalier	(2)	0.51
Oderbrucker	(2)	73.69
Tipple	**(2)**	73.04
Vellavia	(2)	41.86
Westminster	**(2)**	35.46

The relationship between pg fungal DNA per ng and percentage of infected grains for barley cultivars is a positive one but it is not significant (R^2= .558, *P* = .088) (Figure 3.7).

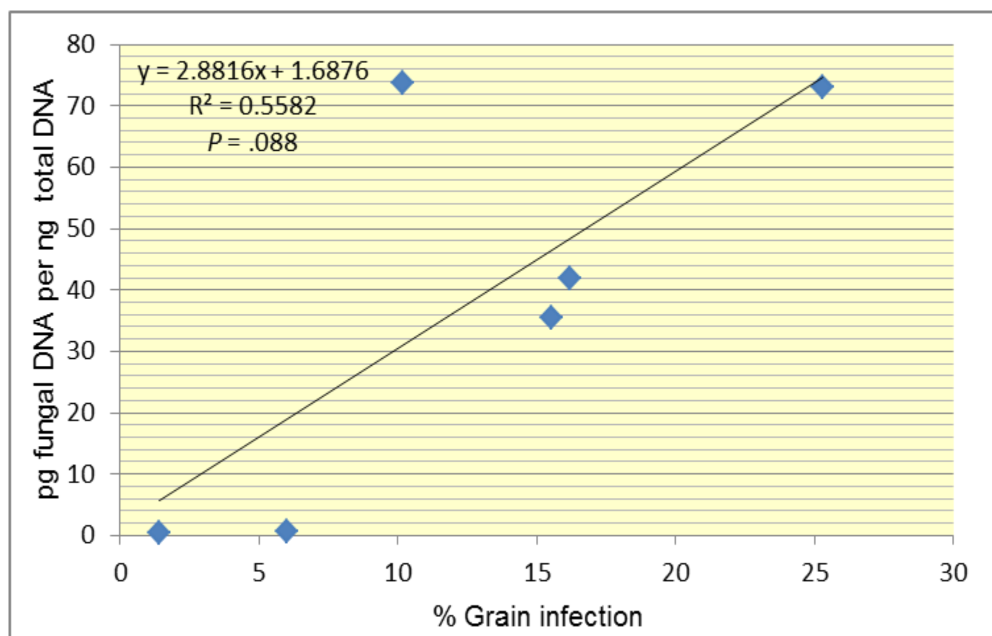


Figure 3.7: Regression between Pg fungal DNA per ng and percentage of infected heads for barley cultivars, field experiment, JIC, 2010.

The results indicated that Chevalier and Armelle varieties have very limited levels of DNA. The limited DNA levels associated with low infection levels around 5% indicated that Chevalier and Armelle varieties have good resistance against the growth of *F. culmorum* in contrast to other barley varieties.

3.3.5 Microorganisms of barley samples.

The fungi from barley grains were isolated and identified by plate culturing and morphological appearance. Identification was confirmed by conidia morphology with a light microscope (magnification x400). The data were obtained by counting the number of fungal colonies growing from barley grains harvested in the 2009 field trial. The results indicated that all samples were contaminated with *Fusarium spp.* and *Alternaria* fungi*.* The results showed differences among varieties with higher levels of *Fusarium spp.* isolated from Dore, Vellavia, Union, Asplund and Westminster varieties. In addition to *Fusarium*, the fungus *Alternaria* was the most dominant fungus in all samples (Table 3.10). Surface sterilization reduced the number of fungal colonies but did not eliminate the counts.

Table 3.10: Microorganisms from harvested grains on PDA media.

Variety (2 or 6 row)		Without sterilization								sterilized with sodium hypochloride						
		Fusarium	*Alternaria*	*Trichothecium*	*Mycelia sterlia*	*Mucor*	*Penicillium*	Bacteria	Total fungi	***Fusarium***	*Alternaria*	*Trichothecium*	*Myceliastelia*	*Mucor*	Bacteria	Total fungi
Armelle	(2)	**8**	4	1	2				15	**3**	8		1			12
Asplund	(6)	**9**	7	2		1			19	**5**	10		3			18
Bigo	(6)	**7**	4	2			5		18	**6**	5	2	4			17
Chevalier	(2)	**6**	11	1					18	**4**	10		1			15
Dore	(6)	**13**	4		2				19	**6**	8		2			16
G d velay	(2)	**6**	7	2	1	2	1	1	19	**1**	9	2	3		1	15
Hannchen	(2)	**8**	9	1					18	**4**	5	1	5			15
Loibichl	(2)	**8**	3	3					14	**2**	4	4	6			16
Nottingham	(2)	**5**	6	4	3		1		19	**3**	9		5		1	17
Oderbrucker	(6)	**8**	6	2	1	1			18	**9**	3		3			15
Plumage	(2)	**7**	5	1	3				16	**5**	6	3			3	14
Tipple	**(2)**	**8**	9	3	1				21	**4**	10		2			16
Union	(2)	**9**	3	1	2	2			17	**7**	3		4	1		15
Vellavia	(2)	**10**	7		1	2			20		5	5	5			15
Westminster	**(2)**	**10**	7						17	**6**	9				1	15

The relationship between the percentage of infected plants and the number of *Fusarium spp*. colonies isolated from infected barley grains is significant (R^2=.393, P = .012) (Figure 3.8). One sample (Dore) showed a higher level of infection than would be expected from the relationship but may be an anomaly as the experiment only generated a single data set.

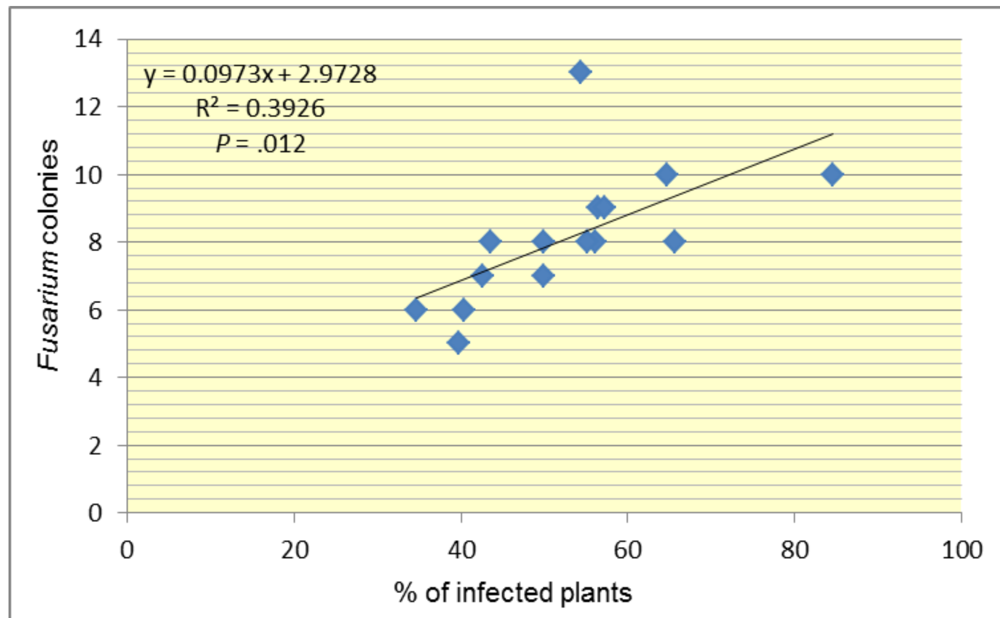


Figure 3.8: Regression between the percentages of infected plants and *Fusarium* colonies number isolated from barley grains at harvest, field experiment, 2009.

3.3.6 Mycotoxin analysis.

Assessment of *F. culmorum* infected barley heads demonstrated mycotoxin contamination of all barley varieties. The results from mycotoxin analysis from 2009 field samples and 2010 glasshouse experiments using ROSA and HPLC-MS methods are presented in Tables 3.11- 3.13.

Table 3.11: Levels of DON (ppb) extracted from barely samples harvested at Nafferton farm, Newcastle, 2009 using the ROSA method.

Varieties/ Row type		DON (ppb)
Hannchen	(2)	333
Gloire du velay	(2)	366
Chevalier	(2)	433
Nottingham	(2)	433
Tipple	**(2)**	**533**
Union	(2)	533
Westminster	**(2)**	**533**
Asplund	(6)	633
Plumage	(2)	633
Armelle	(2)	666
Dore	(6)	733
Vellavia	(2)	766
Oderbrucker	(2)	800
Bigo	(6)	1533
Loibichl	(6)	1900

One-way parametric ANOVA (Cosine)			
P	df	F	CV
.607	14	.853	.86

ANOVA analyses of variance, P probability, df degree of freedom and CV coefficient of variation

(0-1000 ppb Accepted, 1001-1250 ppb Retest, >1250 ppb Rejected)

Table 3.12: Levels of DON (ppb) extracted from barely samples harvested in JIC, Norwich, 2010 using HPLC-MS and ROSA methods.

HPLC-MS			ROSA		
Variety/ Row type		DON (ppb)	Variety/ Row type		DON (ppb)
Chevalier	(2)	1555.20	Chevalier	(2)	650
Armelle	(2)	5602.50	**Tipple**	**(2)**	**2200**
Westminster	**(2)**	**6845.80**	**Westminster**	**(2)**	**2200**
Oderbrucker	(6)	13514.40	Armelle	(2)	2975
Tipple	**(2)**	**14534.40**	Oderbrucker	(6)	3400
Vellavia	(2)	15561.20	Vellavia	(2)	4475

HPLC-MS (Log)					ROSA			
One-way parametric ANOVA					One-way parametric ANOVA			
P	df	F	LSD	CV	P	df	F	CV
< .001	5	23.164	3955.156	.66	. 166	5	1.79	.79

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference and CV coefficient of variation

(0-1000 ppb Accepted, 1001-1250 ppb Retest, >1250 ppb Rejected).

Table 3.13: Levels of DON (ppb) extracted from barely samples harvested from glasshouse plants in Sunderland, 2010 using the ROSA method.

Variety/ Row type		DON (ppb)
Chevalier	(2)	733
Tipple	**(2)**	**800**
Plumage	(2)	1000
Westminster	**(2)**	**1400**
Oderbrucker	(6)	2033
Vellavia	(2)	2600
Armelle	(2)	3666

One-way parametric ANOVA (Cosine)			
P	df	F	CV
.682	6	.662	1.02

ANOVA analysis of variance, P probability, df degree of freedom and CV coefficient of variation

(0-1000 ppb Accepted, 1001-1250 ppb Retest, >1250 ppb Rejected).

The relationship between the levels of DON determined by the ROSA and by HPLC methods is not significant (R^2= 0.555, P = .089) (Figure 3.9).

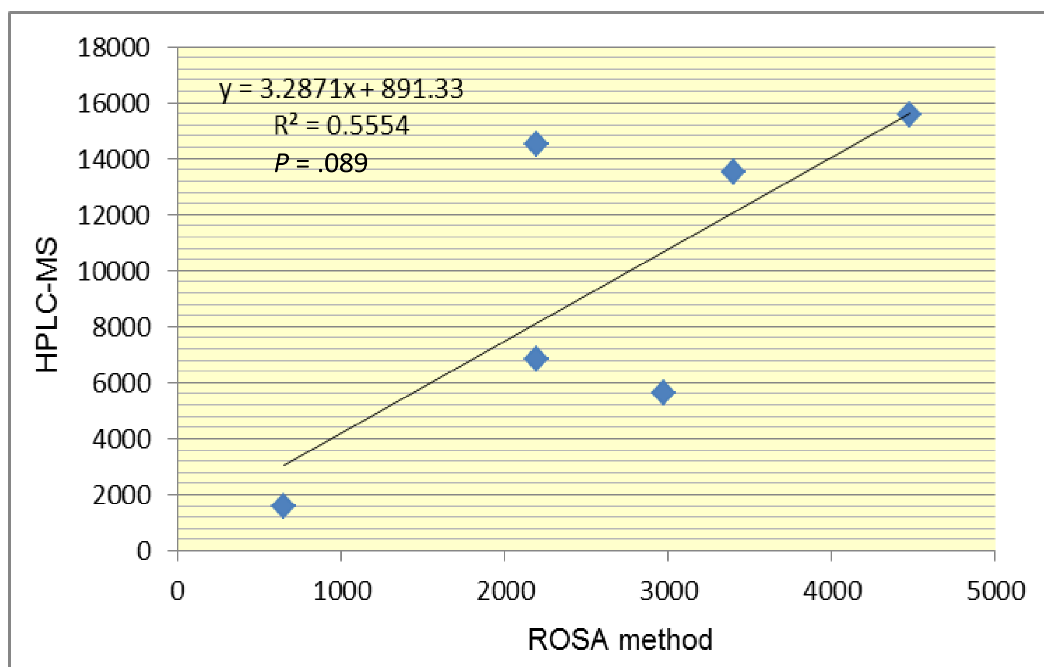


Figure 3.9: Regression between ROSA and HPLC-MS methods for mycotoxin analysis.

Statistical analysis revealed no significant differences between barley varieties when analysed using the ROSA method (Tables 3.11-3.13). However, the HPLC-MS method revealed significant differences between barley varieties (F (5, 23) = 23.164, $P < .001$) (Table 3.12).

Using the ROSA method the levels of DON mycotoxins recorded for barley samples harvested in 2009 reached rejection levels in Bigo and Loibichl varieties only. However, the results of ROSA tests carried out for barley samples harvested at JIC in 2010 showed higher levels of DON in most barley samples and that these exceeded the rejection level in all varieties except Chevalier. On the other hand, the calculated values of DON using HPLC-MS for the same barley samples indicated that all samples analysed contained DON levels exceeding the rejection levels. Nevertheless, lower DON levels were recorded for Chevalier barley compared to other barley cultivars (Table 3.12).

Overall the ranking of DON levels in barley samples from both methods are approximately the same.

Interestingly, the results using the ROSA method recorded lower DON levels in Tipple barley grown under both field and glasshouse conditions than the HPLC method. Armelle barley which presented lower disease levels showed higher DON levels than Tipple. Interpreted data show that the HPLC-MS method gave an approximate compatible ranking sequence with visual disease symptoms. Furthermore, the results also showed that Plumage barley also has acceptable levels of DON mycotoxins in addition to Chevalier barley.

Overall, the highest DON levels were found in Bigo, Loibichl, Oderbrucker, Tipple and Vellavia. The low levels of visual disease symptoms in Chevalier barley combined with the low *Fusarium* DNA and DON levels indicate that this is the most resistant variety against FHB disease compared to other barley cultivars whilst inducing lowest DON levels.

A regression analysis indicated that DON levels are significantly related to the pg fungal DNA per ng (R^2= 0.724, *P* = .032) (Figure 3.10).

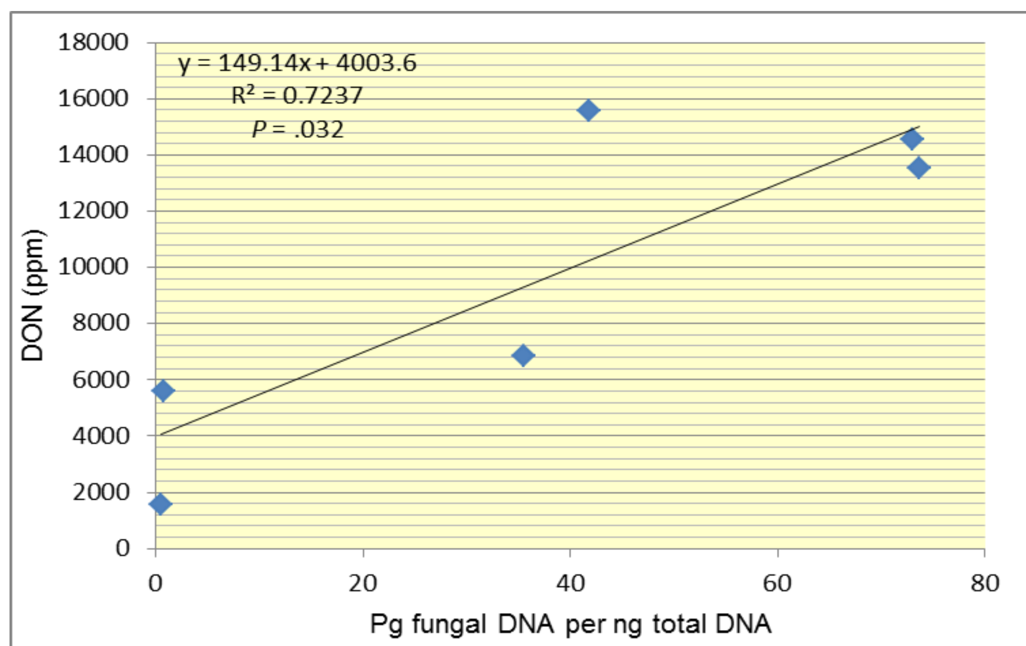


Figure 3.10: Regression between DON levels determined using HPLC-MS and fungal DNA for barley varieties, field trial, JIC, 2010.

3.3.7 Barley growth characteristics.

3.3.7.1 Growth characteristics, field trials, 2009 (F 2009).

Barley growth indicators of plant height, tiller number, dry weight, grain yield, 1000 grain weight and nitrogen content in seeds for all barley cultivars of preliminary field growth trials in 2009 are given in Table 3.14. Statistical analysis revealed significant differences between varieties for all growth characteristics.

Plant height varied considerably over all varieties. In general, Tipple, Dore and Westminster are short varieties (55-70 cm) while Hannchen, Loibichl, Oderbrucker, Gloire du Velay, Nottingham, Chevalier, Bigo and Plumage are tall varieties (91-106 cm).

Armelle, Loibichl, Nottingham, Westminster and Vellavia cultivars produced a greater tiller number (≥4 tillers per plant) compared to other barley cultivars. The six-row barley cultivars produced lower numbers of tillers compared with two-row barley cultivars (<3).

Dore, Tipple, Union, Oderbrucker, Asplund and Bigo had less total dry weight (<3 g) compared to other barley varieties while Gloire du velay, Vellavia, Loibichil, Plumage and Nottingham had greater dry weight (3.75-4.88 g per plant).

Seed yield showed that the modern variety Westminster produced the highest yield (over 4 g per plant). Gloire du velay, Plumage, Armelle, Nottingham and Vellavia varieties also produced high yield above 3 g per plant.

The results presented in Table 3.14 also indicate that six-row barley cultivars (Asplund, Dore, Bigo and Oderbrucker) produced lower yield per plant in comparison to two-row historic and modern barley cultivars.

One thousand grain weight revealed that the seeds of six-row barley cultivars were of smaller size compared to two-row barley cultivars as detailed in Table 3.14. Statistical analysis revealed significant differences in 1000 grain weight between varieties ($F\,(14, 30) = 30.436$, $P < .001$). Modern varieties Tipple and Westminster

and Gloire du velay had significantly higher seed mass, over 49 g per 1000 seeds, compared to six-row varieties and most historic two-row varieties. Historic varieties Vellavia, Chevalier and Plumage also showed high masses over 46 g per 1000 seeds. These figures are comparable to benchmarks for modern agronomic production (HGCA 2005) and for malting requirements.

Relationship between barley growth characteristics and grain yield indicated that there was a significant relationship between tiller number and grain yield (R^2 = 0.659, *P* .006) and between tiller number and 1000 seed weight (R^2= 0.433, P < .001). However, a poor regression was found between plant height and seed yield (R^2= 0.011, *P* .234) and also between plant height and 1000 seed weight (R^2= 0.057, *P* .113). The relationships between tiller number and grain yield or between plant height and grain yield are shown in Figures 3.11 and 3.12.

Powdery mildew disease appeared on most barley varieties at seedling stage, however there was no clear effect on barley growth. A preliminary analysis to evaluate the validity of an ANCOVA test indicated that the interaction between the incidence of mildew and varieties in the prediction of the grain yield is not significant (F (14, 15) = 1.822, p = .130). It was thus appropriate to proceed to the ANCOVA analysis. The relationship between the covariate (mildew) and the dependent variable (grain yield) determined using ANCOVA is not significant (F (1, 27) = .107, p = .746) indicating that there is no significant relationship between mildew disease and grain yield. Furthermore, the relationship between the percentage of infected area covered with mildew and grain yield is not significant (R^2 = .013, P = .464) (Figure 3.13). Overall, ANCOVA results combined with a poor relationship between mildew and grain yield indicated that mildew disease had little influence on grain yield while barley varieties had the most influence on grain yield which varied between 1g in Dore to 4.4g in Westminster.

A significant variation in the nitrogen content was observed in seeds from different barley cultivars (Table 3.14) (F (14, 30) = 7.547, P < .001). When total N is considered, the results showed that a group of six barley varieties including Westminster, Tipple and Chevalier had significantly lower levels of nitrogen (≤ 1.32%) compared to the six- row barley varieties particularly Asplund and Dore

which had relatively high levels of nitrogen above 1.7%. Other historic varieties had acceptable N content for malting purposes.

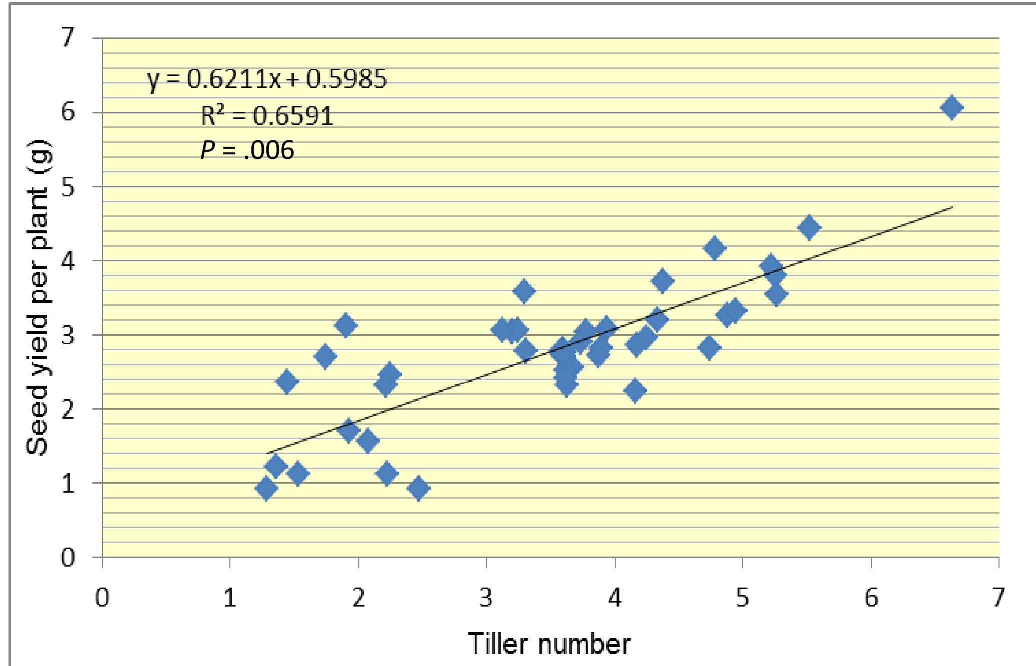


Figure 3.11: Regression between tiller number and grain yield under field conditions, 2009.

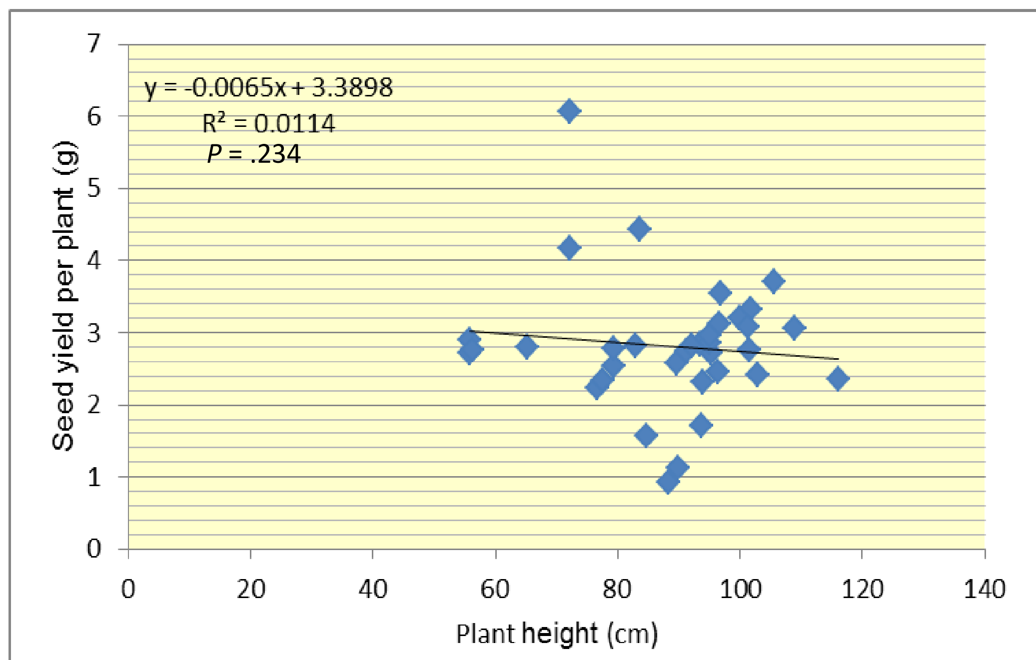


Figure 3.12: Regression between plant height and grain yield under field conditions, 2009.

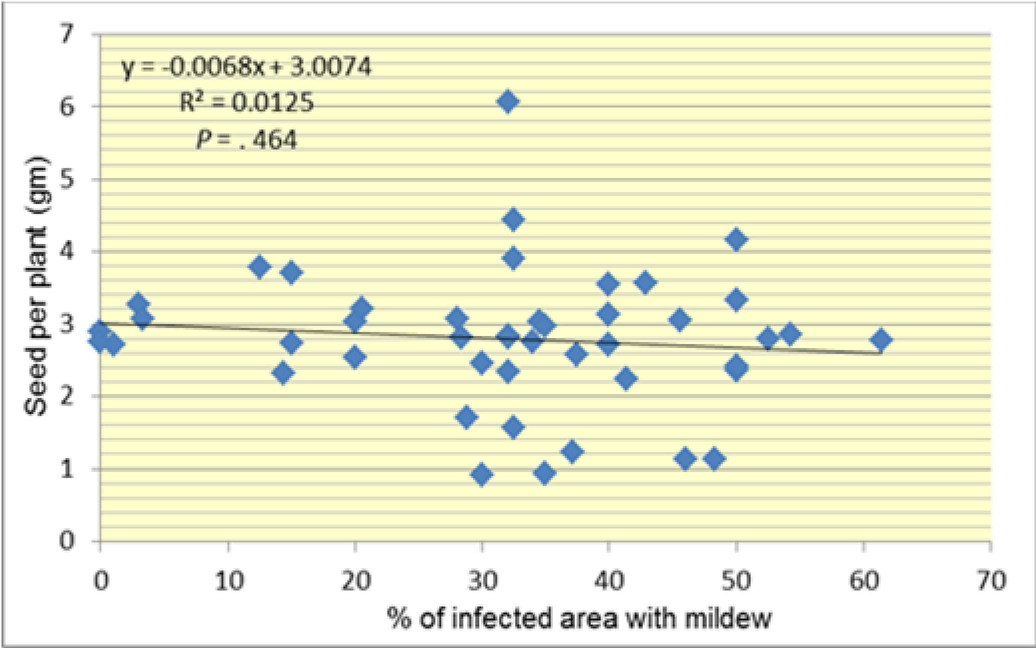


Figure 3.13: Regression between the percentages of infected area with mildew and grain yield under field conditions, 2009

Table 3.14: Barley growth characteristics, field trials, 2009 (F 2009).

Variety (2 or 6 row)		Plant height (cm)	Tiller number	Dry weight (g)	grain yield per plant (g)	Weight of 1000 grains (g)	Percentage of nitrogen
Armelle	(2)	81.98	4.24	3.37	3.34	41.6	1.47
Asplund	(6)	87.68	2.26	2.30	1.21	33.68	1.85
Bigo	(6)	102.69	1.70	2.37	2.73	36.84	1.55
Chevalier	(2)	101.92	3.71	3.59	2.75	47.07	1.32
Dore	(6)	57.90	1.39	0.84	1.09	26.28	1.96
Gloire due velay	(2)	99.48	3.25	3.75	3.22	52.46	1.40
Hannchen	(2)	91.46	3.81	3.00	2.71	41.82	1.69
Loibichl	(2)	94.08	4.39	3.87	2.88	43.98	1.63
Nottingham	(2)	99.49	4.84	4.88	3.35	44.39	1.60
Oderbrucker	(6)	94.64	2.13	2.24	2.16	36.17	1.56
Plumage	(2)	106.59	3.43	4.75	3.27	47.83	1.61
Tipple	**(2)**	**55.93**	**3.65**	**1.69**	**2.79**	**49.06**	**1.31**
Union	(2)	77.87	3.80	2.17	2.37	40.90	1.55
Vellavia	(2)	85.56	5.12	3.78	3.65	46.67	1.46
Westminster	**(2)**	**69.85**	**4.91**	**3.05**	**4.34**	**49.17**	**1.22**

Plant height				Tiller number				Dry weight				Grain per plant			
One-way non-parametric ANOVA				One-way non-parametric ANOVA				One-way non-parametric ANOVA				One-way non-parametric ANOVA			
P	df	Chi-square	CV	*P*	df	Chi-square	CV	*P*	df	Chi-square	CV	*P*	df	Chi-square	CV
< .001	14	42.153	0.18	.001	14	36.393	0.35	.001	14	37.48	0.39	.001	14	35.450	0.34

1000 seed weight					Nitrogen content in barley seeds				
One-way parametric ANOVA					One-way parametric ANOVA				
P	df	F	LSD	CV	*P*	df	F	LSD	CV
< .001	14	30.436	1.352	0.17	< .001	14	7.547	0.078	0.14

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference and CV coefficient of variation

3.3.7.2 Effect of FHB on barley growth.

Barley growth indicators of plant height, tiller number and dry weight for all barley cultivars at harvest from glasshouse samples in 2010 (G 2010) at (GS 92) are given in Table 3.15. It is concluded that FHB disease had no significant effect on plant height, tiller number and dry weight of barley.

Plant height varied considerably over all varieties. In general, Westminster, Tipple and Armelle are short varieties (60-68 cm) while Oderbrucker, Chevalier and Plumage are classified as tall varieties (above 70 cm). Statistical analysis revealed significant difference between varieties (F (6,126) = 41.661, P < .001 for control plants) but there was no significant effect of FHB infection on plant height (P = .552) (Table 3.15a).

Plant dry weight results revealed that Plumage, Vellavia and Westminster produced greater dry weight compared to other barley varieties. This depends on the plant height and tiller number. Statistical analysis revealed significant differences between varieties (P < .001) but there were no significant differences between infected and uninfected plants (P = .368) (Table 3.15b).

Tiller number results indicated that Westminster, Tipple and Vellavia cultivars produced a greater tiller number (≥ 4-7 tillers) compared to other barley cultivars (Tables 3.15b). Statistical analysis revealed that the combination between varieties and FHB treatment was significant (F (6, 126) = 8.066, P < .001). This indicated that the combined factors of variety and FHB infection had an effect on tiller number of barley, ie, the tiller number in uninfected plants and in infected plants are not the same. There were also significant differences between varieties (F (6, 126) = 14.777, P < .001) but there was no significant effect of FHB on tiller number quantified in harvested plants P = .283. Westminster and Vellavia had significantly greater tiller number compared to other barley varieties (Table 3.15c).

Overall the Regression between FHB incidence and plant height is negative and significant (R^2= .324, P < .001). However, the relationship between FHB incidence and tiller number is positive and significant (R^2= .165, P < .001) (Figures 3.14 and

3.15).

Mildew was prevalent on plants grown under glasshouse conditions. To know whether powdery mildew disease affects the growth characteristics of barley, a preliminary analysis to evaluate the validity of an ANCOVA test indicated that the interaction between the incidence of mildew and varieties in the prediction of plant height is significant (F (5, 57) = 3.449, p = .009) which indicated that differences on plant height vary as a function of the covariate. Significant interaction indicated that the results from ANCOVA are not meaningful and ANCOVA should not be conducted. However, the interaction between the incidence of mildew and varieties in the prediction of the number of tillers and dry weight are not significant (F (5, 57) = .885, p = .497 and F (5, 57) = .928 P = .470 respectively). It was thus appropriate to proceed to the ANCOVA analysis.

ANCOVA analysis indicated that the relationship between the covariate (mildew) and the dependent variables (number of tillers and dry weight) are not significant (F (2, 62) = 1.901, p = .173 and F (2, 62) = .236, p = .629 respectively) indicating that there is no significant effect of mildew disease on number of tillers and dry weight of barley. Overall, ANCOVA results indicated that mildew disease had no effect on the number of tillers and dry weight while barley varieties had more influence on the number of tillers and dry weight.

Table 3.15: Characteristics of barley growth at harvest under control and infected conditions, glasshouse, 2010.

Barley varieties (2 or 6 row)

a- Plant height

	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	**Tipple (2)**	Vellavia (2)	**Westminster (2)**
Control	65.2	71.25	82.6	107.35	**61.65**	70.7	**60.5**
Infected	66.24	92.08	86.01	85.17	**68.61**	71.55	**60.05**

b- Dry weight

	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	**Tipple (2)**	Vellavia (2)	**Westminster (2)**
Control	1.83	2.70	2.99	4.12	**2.13**	4.43	**3.67**
Infected	2.39	2.60	2.67	3.59	**2.44**	3.47	**2.62**

c- Tiller number

	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	**Westminster (2)**
Control	2.8	6.3	4.35	3.25	**4.65**	7.8	**8.15**
Infected	6.08	3.65	4.34	4.37	**6.63**	7.76	**5.11**

Plant height

		One-way parametric ANOVA (SQR)				
		P	df	F	LSD	CV
Control	V	< .001	6	41.661	7.026	.20
		One-way non-parametric ANOVA				
		P	df	(Chi-square)		
Infected	V	< .001	6	60.112		
		One-way parametric ANOVA				
		P	df	F		
	T	. 552	1	.355		

Dry weight

		One-way non-parametric ANOVA			
		P	df	Chi-square	CV
control	V	< .001	6	48.439	.32
Infected	V	< .001	6	31.411	
	T	.368	1	.810	

Tiller number (Log)

	Two-way parametric ANOVA (Log)				
	P	df	F	LSD	CV
V	< .001	6	14.777	3.176	.53
T	.283	1	1.163		
V*T	< .001	6	8.066		

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference, CV coefficient of variation, V between varieties and T FHB treatment

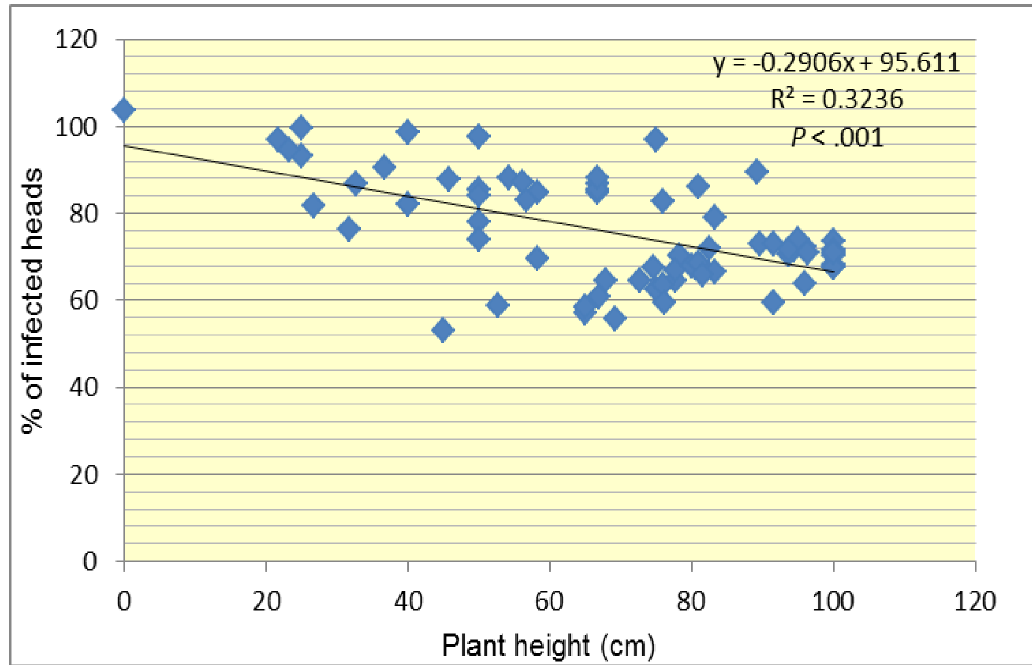


Figure 3.14: Regression of percentage head infection by FHB with plant height for historic and modern barley cultivars at harvest, glasshouse experiment 2010.

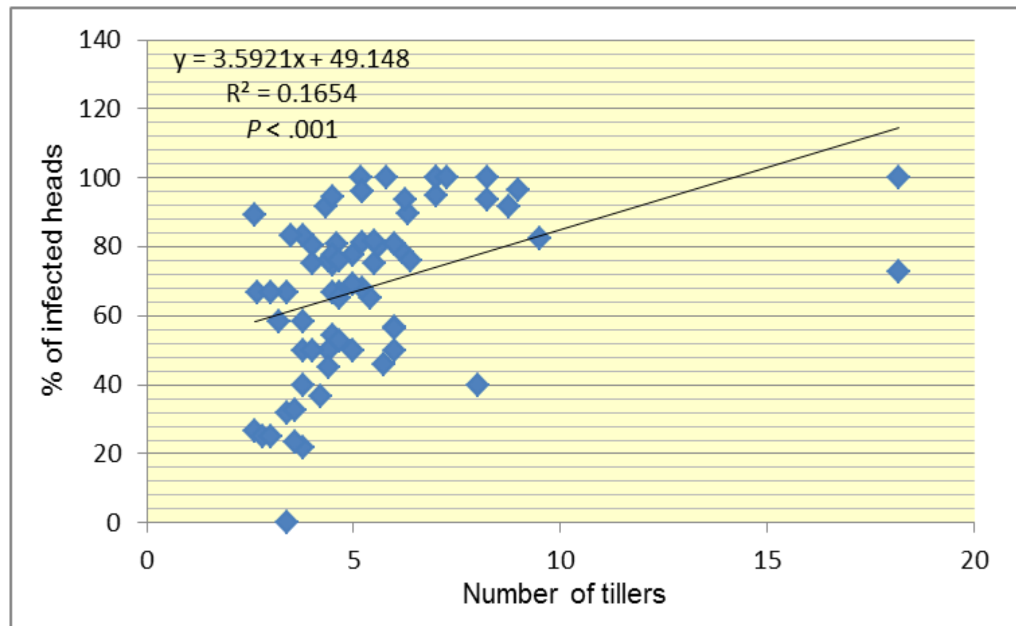


Figure 3.15: Regression between percentage head infection by FHB with tiller number of historic and modern barley cultivars at harvest, glasshouse experiment (2010).

3.3.7.3 FHB and barley grain yield (G 2010).

The effect of FHB on barley grain yield is shown in Table 3.16. The yield per plant ranged from 1.41g in Chevalier barley to 3.17g in Oderbrucker under infected glasshouse conditions. Results from 2010 under glasshouse conditions for barley varieties at GS 92 indicated that Westminster and Vellavia produced the highest yields in comparison with other varieties for uninfected, control plants.

Statistical analysis revealed significant differences between varieties (F (6, 63) = 13.802 and 11.632, $P < .001$) for plants grown under infected conditions and control plants respectively. There was also a significant effect of FHB incidence on barley grain yield in the harvested plants with all varieties producing lower grain yield under infected compared to uninfected conditions. In general, Westminster variety and the historic varieties Vellavia, Plumage and Oderbrucker produced greater yields under uninfected conditions compared to other barley varieties (Table 3.16).

Overall, the results of barley growth suggest that both Westminster and Vellavia cultivars produced more tillers and higher grain yield compared to other barley cultivars. However, both were susceptible to FHB disease. The reduction in yield in infected plants compared to uninfected control plants varied between 13% for Chevalier and 59% for Westminster.

To know whether the powdery mildew disease appearance on most historic barley varieties has an effect on barley grain yield, a preliminary analysis to evaluate the validity of an ANCOVA test indicated that the interaction between the incidence of mildew and varieties in the prediction of the grain yield is not significant (F (5, 57) = .102, p = .991). It was thus appropriate to proceed to the ANCOVA analysis. ANCOVA analysis indicated that the relationship between the covariate (mildew) and the dependent variable (grain yield) is not significant (F (1, 62) = .011, p = .917). This shows that there is no relationship between the mildew disease and the grain yield variable. ANCOVA results indicated that mildew disease had little effect on the grain yield while barley varieties had more influence. The grain yield means for the seven barley varieties ranged between 1.41g in Chevalier to 3.17 in

Oderbrucker.

Table 3.16: Grain yield (g) per plant for barley varieties grown under control and infected conditions, glasshouse, Sunderland, 2010.

Variety/ Row type		Control	Infected	% Reduction in yield
Armelle	(2)	2.51	1.99	21
Chevalier	(2)	1.62	1.41	13
Oderbrucker	(6)	4.27	3.17	26
Plumage	(2)	4.49	2.69	40
Tipple	**(2)**	**3.54**	**2.07**	**41**
Vellavia	(2)	4.52	1.86	59
Westminster	**(2)**	**4.67**	**2.65**	**43**

		One-way parametric ANOVA				
		P	df	F	LSD	CV
Control (Logit)	V	< .001	6	11.632	.802	.44
Infected	V	< .001	6	13.802	.456	

	One-way non-parametric ANOVA		
	P	df	Chi-square
V	< .001	6	56.538
T	< .001	1	38.909
	T test-paired		
	P	df	t
T	.005	6	4.308

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference, CV coefficient of variation, V varieties and T FHB treatment

3.4 Discussion.

The hypothesis stated for this investigation was that there would be variation in susceptibility to mildew and *Fusarium* amongst barley varieties, potentially indicating new sources of resistance for future breeding. To test this, selected historic and modern barley varieties were compared for their susceptibility to mildew and FHB and its mycotoxin contamination in relation to their growth features.

The results of mildew infection from field experiments, 2009 indicated that the level of symptoms differed between varieties. In the first assessment, Armelle, Bigo and Chevalier historic varieties had the highest levels of mildew infection. Nevertheless, mildew symptoms disappeared gradually as the plants matured.

The high levels of mildew infection at the first assessment may be related to high humidity around the lower leaves arising from the soil and weeds. However, further assessments indicated that mildew disease disappeared gradually and there were low levels or no symptoms of mildew on the upper leaves for all varieties. These results may be related to plants becoming taller so taking the leaves away from the ground and weeds. The high levels of mildew recorded during the first assessments between May and July are in agreement with Last (1955) who observed that mildew infection levels increased between the end of May and mid-July due to environmental conditions which facilitate spore germination and result in mildew symptoms appearing. The results are also in agreement with Carver (1986) who considered that Vellavia barley cultivar is resistant to pathogen penetration under field conditions. However, the results are in disagreement with Carver (1986) who considered that Gloire du velay is highly field resistant as high mildew levels were observed in this variety.

Results from glasshouse experiments in 2009 showed high levels of mildew infection. The results from the first assessment were a similar indicator of resistance or susceptibility among varieties as seen in the field. Mildew infection under glasshouse conditions became heavier during the growth period and contributed to the demise of most barley varieties from aphid infection.

In spite of a heavy mildew infection levels, modern varieties Westminster and Tipple and the historic variety Plumage showed high resistance against this disease. However, Chevalier and other historic varieties were very susceptible (Table 3.2).

These results do not agree with those from Jones and Davies (1985) who indicated partial resistance against mildew in Chevalier, Gloire due velay, and Loibichl historic varieties at the adult plant stage. Possible explanation for this may be that they are different accessions from those in the study.

The results of mildew infection from different barley cultivars under glass house conditions in Sunderland in 2010 gave similar results showing that Chevalier had the highest levels of mildew infection at all dates of assessment. In general, the historic varieties Chevalier, Vellavia, and Oderbrucker showed high levels of mildew infection compared to modern barley varieties and Plumage historic barley variety which could be related to modern varieties (Tipple and Westminster) possessing the mlo gene (HGCA, 2010).

Observations made in this study indicated that glasshouse conditions were more favourable for mildew disease development which resulted in high levels of mildew in most historic barley varieties. This may be related to environmental conditions which are different to field conditions. It appeared that Chevalier historic barley variety is particularly susceptible to mildew disease under glasshouse conditions and at early stages of field growth.

Inoculation of barley with *Fusarium culmorum FU42* pathogen resulted in disease symptoms that were visible on heads after two weeks. The incidence of *F. culmorum* pathogen responsible for FHB disease development was different among barley cultivars. All results indicated that Chevalier, Armelle and Plumage historic two-row barley cultivars had lower levels of FHB disease compared to the two-row modern barley cultivars Westminster and Tipple. Low levels of FHB disease were also recorded on plants grown under control conditions (uninfected plants) in glasshouse experiments, 2010 (Table 3.5). This may be due to other *Fusarium* species rather than *F. culmorum* causing FHB infection.

Increases in infection levels recorded in the period until harvest could be attributed to the environmental conditions at that time. Temperature and humidity affect the progression of FHB disease. The results from these experiments agree with Vigier and Bourgeois (2005) who indicated that FHB barley infection was initiated in June and that its infection development in July and August are due to DON accumulation.

The outcome of FHB disease level from the JIC field experiment 2010 confirmed the results of the 2009 trial, with similar differences between varieties (Table 3.6). Different geographical locations were associated with the two field trials and may have incurred different environmental conditions between the north and the south UK locations. Overall, all field and glasshouse experiments for both years and for different geographical locations indicated that Chevalier barley had consistently lower levels of infection with FHB which suggests that Chevalier barley is the most resistant cultivar against FHB disease. A conclusion from these results is that breeding programs could usefully focus on the Chevalier barley cultivar as showing best resistance against FHB disease. This observation then leads to the question of what factors effect resistance?

A higher incidence of natural *Fusarium* infection was noted on six-row barley heads compared with two-row barley cultivars (Table 3.4) during the initial field studies, 2009 (F 2009). However, the FHB disease symptoms in the field could result from infection by different *Fusarium* species other than *Fusarium culmorum*. This is in agreement with Takeda and Heta (1989) who reported the relationship between morphological characteristic of heads and FHB resistance in barley noting that two-row barley cultivars are more resistant to FHB disease compared to six-row barley cultivars. Thus could be related to the greater aeration in two-row barley heads being less suitable for fungal development (Mesfin *et al,* 2003) and also to a lower level of Type II resistance in six-row barley varieties. Foroud and Eudes (2009) proposed that resistance in two-row barleys is related to a QTL that is associated with the *Vrs1* locus which controls head type. Langevin *et al* (2004) proposed that six-row barley has a moderate Type II resistance and the fungus was frequently observed to move externally from one floret to another within the dense head without penetrating the rachis.

Cleistogamous seed type is another important characteristic for resistance. For example, Japanese two-row barley varieties are cleistogamous and presented high resistance against FHB, whereas six-row varieties are chasmogamous and are moderately resistant or susceptible to FHB. In general, most two-row barley cultivars are cleistogamous whereas most six-row cultivars are chasmogamous (Yoshida *et al*, 2001). It has been found that the size of lodicule is larger in chasmogamous cultivars compared to cleistogamous cultivars especially at the white anther stage associated with more cell division activity in chasmogamous types. (The lodicules are the two diminutive bodies lying between the lemma and the ovary base in the grass floret which expand rapidly at the time of anthesis to lever away the rigid lemma allowing anthers and stigmas to emerge). The larger size of the lodicule may perhaps be responsible for pushing open the lemma and thereby opening the floret. It is proposed that cleistogamy provides a means of escape from FHB infection (Nair *et al*, 2010). Two genes *cly1* and *cly2* are the genetic control of cleistogamy type in barley (Turuspekov *et al*, 2004).

In general, the most critical time for *Fusarium* infection and subsequent mycotoxin accumulation in barley differs among cultivars and appears to be associated with the flowering time. A study conducted by Yoshida *et al* (2007) for two-row cleistogamous cultivars in Japan, revealed that the infection occurring after the extrusion of spent anthers would be much more important than infection around anthesis. On the other hand, cleistogamous cultivars had higher levels of mycotoxins when inoculated 10 or 20 days after anthesis compared with plants inoculated at anthesis, but chasmogamous cultivars accumulate more mycotoxins when inoculated at anthesis (Yoshida *et al*, 2007). They suggested that the optimal timing for chemical control of FHB and mycotoxin contamination in barley depends on the cultivar which is probably associated with the flowering type. Late infection, even without visible symptoms of FHB symptoms, was also associated with the risk of mycotoxin contamination which suggests that there is an inconsistent relationship between mycotoxin concentration levels and disease appearance.

For cleistogamous cultivars, the efficacy of fungicide applications is expected to be improved by changing the timing of application to be near the extrusion of spent anthers. In Japan, fungicide application to control FHB in barley is usually

performed around the time of anthesis which occurs a few days after the full heading stage (Yoshida *et al*, 2007).

FHB resistance could be also related to hormone signalling, for example gibberellic acid (GA) has important roles on plant life cycle, stem elongation, trichome development, pollen maturation, flower induction, seed development and in regulating seed germination and could be providing resistance mechanisms for different tissues. DELLA proteins are key regulators of GA mediated growth and development promoting resistance to necrotrophs and susceptibility to biotrophs. DELLA dwarfing alleles have been identified in cereals but are not currently used in agriculture (Alvey and Boulton, 2008). (DELLA proteins are highly conserved proteins that repress plant growth. They are present in plant cell nuclei, and in response to gibberellin, become phosphorylated, polyubiquitinated and targeted for degradation by proteasomes).

Differences in flowering dates have been noted among varieties used here in field growth and in glasshouse growth. Plants in 2010 at the JIC field trial and the 2010 glasshouse trials were mist inoculated throughout the anthesis of all varieties and so received comparable exposure to spores. Type II resistance is present in two-row barley varieties unlike six-rowed varieties and other cereals such as wheat (Langevin *et al*, 2004) and so infection rates are a valid reflection of the disease incidence and of the variety susceptibility.

The time of anthesis is regarded as an important stage for *Fusarium* infection in barley. The results of different infection levels may be related to different flowering dates or different periods of flowering between different barley cultivars. Numerous studies conducted by Hill *et al* (2006), Dahleen *et al* (2003), Urrea *et al* (2002) and Klahr *et al* (2007) indicated the effect of heading date and plant height on FHB diseases. However, Zhu *et al* (1999) proposed that there is no association between days to heading and FHB incidence in barley. Nevertheless, data in this study indicate that taller plants have higher resistance.

The duration of anthesis was not assessed for the varieties studied here. However, the method of inoculation (repeated at short intervals throughout

anthesis) would ensure that inoculum was applied at appropriate times for all lines. The majority of heads in each plot would thus have received inoculum at a similar developmental time. Many lines begin to flower when the heads are still in the boot stage when the inoculum cannot reach the heads. The methodology employed is designed to get the inoculum onto the heads as soon after they emerge from the flag boot as possible.

Differences in infection levels are thus unlikely to be due to different applications of inoculum. This is supported by there being no significant correlation between the percentage of infected grains and flowering date (-.592, P = .081) and a poor correlation between the percentage of infected heads and flowering date (-.672*, p = .049). Furthermore, the infection level in Oderbrucker was less than modern varieties and Vellavia historic variety although it was the first variety to produce heads. All these results suggest that there was no effect of repeating inoculations over short intervals and the susceptibility against FHB disease could be attributed to different genotypes between barley varieties.

Varieties may however, differ in their length of flowering period and so receive different doses of inoculum as shown in situation B in Figure 3.16. In situation A equal exposure at different times will lead to similar levels of infection unless resistance factors differ. In situation B different exposures will be received due to different flowering times.

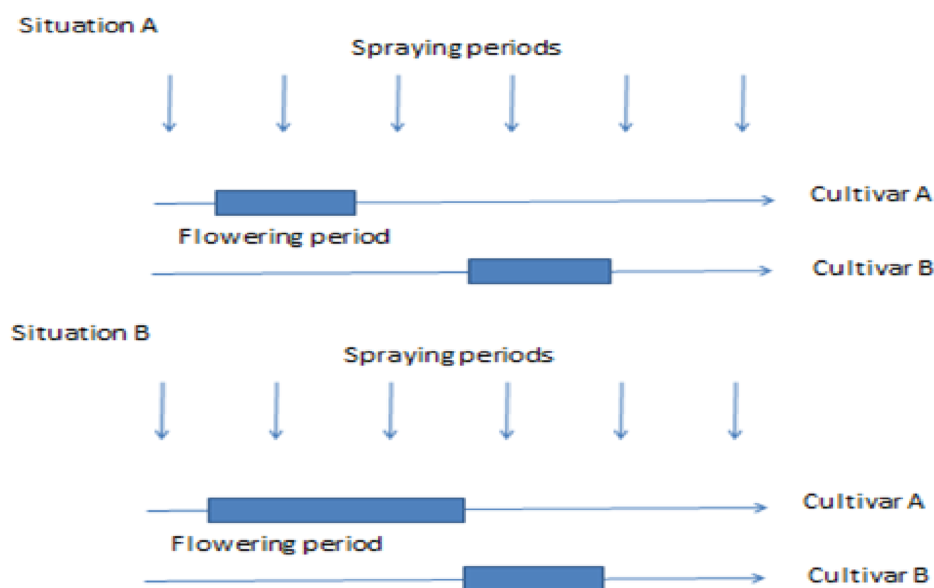


Figure 3.16: Effect of repeating artificial inoculation and flowering period on FHB infection levels.

Overall, resistance against FHB can include different responses and physiology. Different flowering dates could be a cause of different infection levels. It is possible that resistant barley cultivars have a short flowering period that is complete by the time of emergence. This could be considered as escape rather than true resistance but as it is inextricably linked to prevention of conidia reaching the heads it can be seen as a resistance mechanism. Further experiments to identify the flowering times could provide evidence to support this possibility.

Analysis of fungal DNA by real time PCR revealed very limited amounts of fungal DNA (around 0) in Chevalier and Armelle varieties which confirm resistance in these varieties in contrast with other barley varieties. However, the limited DNA levels associated with low infection levels around 5% suggests that these varieties could be contaminated by *Fusarium* species other than *F. culmorum* causing the same FHB symptoms (Figure 3.7).

The common fungi isolated and identified from barley grains were *Fusarium spp.* and *Alternaria* (Table 3.10). The genus *Alternaria* was the most dominant fungus in all samples after *Fusarium,* and the prevalence of *Alternaria spp.* was recorded in

grains at harvest time. *Alternaria* fungus is parasitic to plants with infected grains showing black point symptoms. It is the main fungus in grains especially from late harvest (Hudec, 2007). *Alternaria* fungus also is the most common fungus in malting barley samples. It has the ability to produce alternariol and alternariol monomethyl mycotoxins (Medina *et al*, 2006). Kosiak *et al* (2004) reported that higher levels of *F. graminearum* and *F. culmorum* were found in poor quality grain while acceptable barley grains showed *Alternaria spp.* contamination. However, *Alternaria* and *Fusarium* species have been noted to kill embryos or decrease embryo vigour and in addition have the ability to produce various types of toxic metabolites (Hassan, 1999).

Analysis of *F. culmorum* infected barley heads demonstrated DON mycotoxin contamination in seeds of all varieties. The results confirmed that barley samples had a range of levels of DON and presented lower mean DON levels in barley samples analysed by using ROSA method compared to analysis using HPLC-MS (Tables 3.11- 3.13). Mycotoxins analysis also showed that some barley samples were contaminated with high levels of DON (> 6000 ppm) associated with limited levels of DNA (around 0). These results suggested that other *Fusarium* species in addition to *F. culmorum* may be causing FHB infection. Nevertheless, a positive regression was found between *Fusarium* DNA and DON levels in barley (Figure 3.10) which agree with previous work conducted by Demeke *et al* (2010) who reported a positive correlation between *Fusarium* DNA and DON levels in barley by using real-time PCR assays.

The safe limit of DON for commercial use is set at 1250 ppb and most samples from the preliminary field trials were within this safe limit (Table 3.11) (Salmon and Matthews, 2007). Samples from inoculated plants from JIC and glasshouse experiments in 2010 analysed by ROSA were mostly above this limit (Tables 3.12 and 3.13). Levels analysed by HPLC also exceeded the limit (Table 3.12). These high levels may result from the presence of high levels of *Fusarium* resulting from artificial infection and not be reflective of standard growth conditions in naturally-infected fields.

The results showed that in some cases no FHB disease symptoms were present

but the presence of DON was detected, for example in Chevalier albeit at low levels. These results agree with Hill *et al* (2006) and Liu *et al* (1997) who indicated that there is no correlation between visual symptoms and DON levels in some genotypes. Previous work has suggested that the biosynthesis of mycotoxin is enhanced when the fungus is under stressful conditions such as nutrient deficiency or exposure to antifungal materials produced by the resistant varieties (Nicholson *et al,* 2003).

Further reasons for these differences as suggested by Culler *et al* (2007) related to environmental conditions especially temperature and humidity that affect DON and FHB disease separately. Another study conducted by Cowger *et al* (2009) showed that a period of moisture after barley flowering has a positive effect on both FHB and DON levels in wheat. For this reason a grower is advised to count the effect of prolonged rainy periods post flowering to predict potential infection. Nowadays many breeders evaluate cultivars for FHB resistance and DON depending on the natural rainfall or misting for the period around anthesis. The timing of inoculation also affects both FHB and mycotoxin concentration. For example, in two-row varieties DON levels are mostly higher with inoculation at 10 than at 0 days after anthesis (Yoshida *et al*, 2007).

In general, the use of both ROSA and HPLC-MS methods facilitate the detection of DON mycotoxins even at low levels. The ROSA method for DON is the fastest and simplest for mycotoxins analysis and determines the contamination of cereals grain within just 15-20 min. Its range of detection is 0-5000 ppb DON and a high correlation has been found in other studies between the ROSA test and the results of (GC/MS) analysis (Salmon and Matthews, 2007). However, the present results showed that the relationship between the ROSA and HPLC-GC tests is not significant (Figure 3.9). In general, the ROSA method has been developed for cereals grown under normal condition but not for inoculated samples.

Selected barley varieties were also compared for their growth features in relation to their susceptibility to mildew and FHB incidence. Chevalier barley showed very similar yields from field samples in 2009 to Tipple and similar grain sizes to Tipple and Westminster. Chevalier also had very similar grain nitrogen content to Tipple

(Table 3.14) and was comparable to contemporary expectations (HGCA, 2005) so justifying further study of this variety. Plumage, Armelle, Nottingham and Vellavia also gave good grain yields from field growth. Grain yield and 1000 grain weight were correlated with tiller number however there was no relationship with plant height. Varieties with higher tiller numbers produce greater yields with greater mass compared to varieties with low tiller numbers (Figure 3.11). In spite of the powdery mildew disease appearance on most barley varieties at the seedling stage, there was no clear effect on barley growth. The relationship between mildew and the grain yield is not significant which indicated that there is no effect of mildew disease on grain yield. The reason for this may be related to the decrease in mildew symptoms gradually during growth and their disappearance at the adult stages even on susceptible varieties.

Regression analysis indicated that the tiller number and plant height affect FHB infection levels (Figures 3.14- 3.15). Plant height results are in agreement with Urrea *et al* (2002) who indicated a negative correlation of FHB disease incidence with plant height, days to heading, head angle, head mass and head type. Klahr *et al* (2007) also revealed a negative association between FHB resistance and plant height.

In general, most growers prefer short cultivars since they give higher grain yields with reduced lodging. A study conducted by Voss *et al* (2008) on short wheat cultivars carrying semi-dwarfing allele Rht-D1b (Rht2) showed shorter plants are associated with more than a two fold increase in FHB levels as a result of *F. culmorum* infection. Another study conducted by Gosman *et al* (2009) in high FHB disease density revealed that both Rht-B1b and Rht-D1b dwarfing genes reduce Type I resistance. On the other hand, no influence was found of Rht-D1b on Type II resistance. The study reported here suggests a similar relationship between increased plant height and enhanced FHB resistance in barley.

The results of barley yield indicated that grain weight varied considerably among varieties (Table 3.16). This depends on the tiller number per plant and different grain size among varieties. However, it appeared that FHB disease decreased barley grain yield weight for most barley cultivars causing tombstone and

shrivelling and possibly sterile florets (McMullen *et al*, 1997).

The grain yield in individual experiments is largely influenced by weather conditions. The highest yield production was reported in Westminster and Vellavia (4.67, and 4.52 g per plant) under glasshouse conditions in 2010. The reason for the higher yield productivity in Westminster barley may be related to this variety having a semi-dwarf gene (*sdw1*). This has an important role in reducing plant height and increasing grain yield by enhancing the ability of roots to absorb nitrogen and elements from the soil (Ellis *et al,* 2002; White *et al,* 2009).

The results also showed that Chevalier barley cultivar produced higher yield under field conditions compared with glasshouse conditions. This may be due to Chevalier barley being very susceptible to mildew disease, which occurred more extensively under glasshouse conditions compared to field conditions. The present results agrees with Kozdoj *et al* (2009) who reported that the grain yield per plant of susceptible barley cultivars is considerably lower compared to resistant barley cultivars. The high level of mildew frequently resulted in lower yield in Chevalier and is likely to be related to a reduction in photosynthesis level leading to plant leaves necrosis (Swarbrick *et al,* 2006). This is likely to reduce tiller and grain numbers in heads and grain size (Scott and Griffiths, 1980). Furthermore, decreases in shoot number may be related to the pathogen effects on meristematic activity (Last*,* 1962a).

In summary, it appears that historic varieties are in many cases different from modern varieties of barley in being more susceptible to mildew disease. However, some historic barley varieties have better resistance to FHB compared to modern varieties. This may result from a number of causes including density of foliage and plant height. It may also have genetic causes, possibly due to the presence or absence of the mlo gene conferring specific resistance to mildew via localised necrosis at the point of infection (Wolter *et al*, 1993).

The potential of using historic barley varieties in future barley breeding may be justified when the varieties have comparable yields and malting characteristics such as low nitrogen levels as for example, Chevalier. A propensity for mildew

susceptibility could be overcome if the varieties could be bred with modern varieties hosting the mlo gene for mildew resistance. The potential for resistance to other diseases is a further consideration as limited symptoms of FHB were noted in field and glasshouse trials on some historic varieties in controlled infection studies with FHB.

3.5 Application to hypotheses.

The investigations reported here address the hypothesis and show significant variation in resistance to FHB between barley varieties. The results show that historic barley varieties Chevalier, Plumage and Armelle have better resistance when compared to modern varieties Tipple and Westminster. This investigation addresses the hypothesis that some historic barley varieties have better resistance to FHB than modern varieties, specifically Tipple and Westminster.

Observation on the flowering date indicated that different barley varieties flowering at different times could be considered as a factor affecting the infection level of FHB. However, the results suggest this is unlikely because inoculum was applied throughout the period. The relationship with height and tillering suggests a relationship between resistance and these growth characteristics. The duration of anthesis was not assessed for the varieties investigated. Assessing and comparing the duration of anthesis would be a good future aim to determine this possibility.

Chapter Four

Effect of nitrogen level on barley growth and disease resistance of modern and historic barley cultivars

4.1 Introduction.

4.1.1 Barley growth.

The features and stages of barley growth are well established (Tottman and Makepeace, 1979). However, growth features and seed yield do vary between varieties and specific characteristics are required for malting barley which requires additional processing before use.

Many historic varieties were superseded by varieties more suitable for management, for example, resistance to lodging or suitability for mechanised harvesting or resistance to disease. Other varieties were discarded due to limited yield particularly for feed barley production. Malting barley differs from this to some extent as different criteria apply for example the need for low seed nitrogen and flavour characteristics such as S-methyl methionine which can lead to high dimethyl sulphide levels in beverages.

Standard agronomic management is typically focused on providing suitable nutrients, particularly nitrogen, phosphorous and potassium to encourage growth. In the case of malting barley, nitrogen application must be carefully regulated to control seed nitrogen levels.

Nitrogen is an essential macronutrient for plant growth. It is an inorganic fertilizer which can be assimilated in large quantities to produce strong, green plants associated with healthy growth and high grain yields. However, because malting barley requires low nitrogen levels and because high levels of N addition leads to small grain sizes, lowers yields and increases the incidence and severity of Fusarium head blight, powdery mildew and other diseases (Lauer and Partridge, 1990; Watson *et al* 1958; Lemmens *et al*, 2004; Russell *et al*, 2008) the actual level of addition requires careful management. Malting barley requires 150 - 200 kg N ha^{-1} of nitrogen (Weston *et al*, 1992) while spring barley requires 130 kg ha^{-1}. The time of application of N is from mid-March to GS 59. Early spring application is given to encourage tillering and to obtain adequate ear number m^{-2}. Late spring N application is used to encourage rapid canopy expansion through tiller survival and

sufficient grains per ear. However, later application of N in the season impairs quality due to high grain N (HGCA, 2005).

Modern barley varieties such as Westminster and Tipple have been developed to produce high yields and easy crop handling and for malting quality with good processing characteristics. Good malting barley has low nitrogen levels, generally below 1.7%, and thus requires careful control of soil conditions and fertilizer addition. In general, the response of plants to increasing concentrations of inorganic nitrogen demonstrates an increase in growth rate and gives maximum yield production. Nitrogen (N), phosphorus (P) and potassium (K) are the most important elements that are required by plants. Other elements required are Zn, Mg, Mn, Ca, Cl, Na, Si, C, B, and Fe.

Nitrogen level and the time of its application affect the quality of malt through effects on grain size, protein content, malt extract and enzyme activity. Nitrogen also affects the diastatic power (DP) - the grains ability to break down starches into sugars. For example, DP increases but sugar extracts decrease with increasing nitrogen fertilizer application. Protein content affects malt quality with high levels having a negative effect by decreasing malt extract, and a positive effect by increasing DP (Qi *et al*, 2005). The level of DP is directly related to protein concentration in the grain. Low diastase levels are associated with a low potential for malt extract. Malt extract measures the amount of fermentable sugars. The higher extract levels mean higher alcohol levels can be achieved in fermentation. Chen *et al* (2006) found that nitrogen levels as well as the timing of nitrogen application also affect grain weight and the extract of barley. Both grain weight and sugar extract decrease with increased levels of nitrogen when applied at the booting stage.

Overall, nitrogen application affects tiller number and the number of grains in each head. Applied nitrogen affects grain quality via three mechanisms: grain size, the dilution of starch by storage protein and the variation in maturity of grains between the main stem and tillers (Ellis and Marshall, 1998). A study conducted by Naylor and Stephen (1993) proposed that larger grains have a low nitrogen content in comparison with small grain size and thus are more suitable for malting. In addition

to grain quality, Drew *et al* (1973) found that high levels of applied nitrogen lead to an increase in the number and extension rate of barley lateral roots combined with increasing dry weight.

The range of nitrogen levels in malting barley cultivars should be 1.4-1.7% (which is lower than in feed barleys). In wort production, the enzymes present in malted grains are necessary to digest starch into fermentable sugar chains and are influenced by grain nitrogen level. The main enzymes to do this are α –amylase, β-amylase, protease and β-glucanase. Barley varieties with the highest nitrogen levels have the highest potential to develop β-amylase enzymes. However, lower nitrogen barley varieties are better modified and so produce higher levels of β-amylase and α-amylase through the malting process. The high levels of β-amylase in malt are related to the efficiency of endosperm modification in the malted grain (Agu and Palmer, 1998).

There are different effects of nitrogen deficiency on barley development. For example, Drew *et al* (1979) found that nitrogen deficiency is associated with improvement in leaf chlorosis and the reduction in N content in the shoots. However, Richards and Templeman (1936) reported that nitrogen deficiency has no effect on photosynthetic activity while the leaf area, total number of leaves, tillering rate and succulence rate decreases with nitrogen deficiency. They also reported that light green leaves were associated with nitrogen deficiency. Furthermore, Nanamori *et al* (2008) reported that the level of the sulphur amino acid S methyl methionine (SMM) concentration in malt increases with low levels of nitrogen while the major S-containing amino acids show no appreciable change due to the variation in grain nutrient status. This is important to malting barley in the production of dimethyl sulphide, an important flavour compound.

In general, the level of nitrogen needed to maximize yield and quality in malting barley is 50 kg ha^{-1} and is associated with greater tiller production (Blazewicz *et al*, 2007). Krentos and Orphanos (1979) recommended that 20–40 kg N ha^{-1} can be applied at seeding together with 13–26 kg P ha^{-1} for barley plants and a similar amount of N should be added in late January.

Last (1962b) studied the effect of fertilizers on mildew disease development. The results showed that susceptibility against mildew disease increases with increasing nitrogen fertilizer rates and is related to increased plant growth and humidity which is more favourable to fungal spread. Jensen and Munk (1997) and Sander and Heitefuss (1998) suggested that increasing colony density with increasing N application enhances the ability of colonies to produce more spores.

Phosphorus (P) is also a major nutrient for plant development and yield. Drew and Saker (1978) indicated that phosphate is required during early growth of barley and P applied to a 4 cm depth leads to an increase in lateral root number and extension. Chapin and Bieleski (1982) investigated different levels of phosphorus and found that high P adapted barley cultivars produced more biomass, tillers, leaf production, final leaf size and total shoot weight while root:shoot ratio decreases with high P.

Potassium (K) salts are also important for plant growth with an important role in controlling the turgor in the vacuole. Amtmann *et al* (2008) reported that potassium deficiency reduces the resistance of plants against pathogens.

N, P, K fertilizer application should be carefully managed for the quality of barley products such as malt. These nutrients should be supplied in a balanced form to obtain greater yield and good quality. For malt purposes, the grains should have relatively low protein content, plump grains and high carbohydrate levels. Growers should use adequate quantities of fertilizers to produce healthy crops and high yields. However, this depends on the time of application and environmental conditions (HGCA 2005). A summary of the effect of N, P and K in barley is shown in Table 4.1.

Table 4.1: Nitrogen, phosphorous and potassium requirements for barley and their deficiency and high level effect.

Element	Rang of growth requirement.	Effect of mineral deficiency	Effect of mineral. high level
N	125-200 kg ha^{-1}	-Reduction in number of leaves and tillers -Decreased succulence. -Reduction in respiration rate. (Richards and Templeman, 1936) -Chlorosis improvement (Drew *et al,* 1979).	-Increase mildew disease. -Increase in FHB infection and mycotoxins contamination (Lemmens *et al*, 2004). -Increase protein, hordine, and β-amylase (Qi *et al* 2006; Yin *et al* 2002). -Improve root growth (Drew *et al,* 1973). -Increase dry weight. -Decrease grain plumpness (Naylora and Stephen, 1993) -Reduction in lysine content (Kirkman *et al*, 1982). -Postpone senescence (Sinclair and de Wit, 1975).
P	15-30 kg ha^{-1}	-Reduction in No. of tillers. -Reduction in yield production. (Chapin and Wardlaw, 1988) -Increase mildew level (Last *et al*, 1962).	-Improve root growth. (Drew, 1975) -Decrease protein (Wang *et al*, 2008).
K	35-90 kg ha^{-1}	-Increases plant susceptibility to insects and diseases (Amtmann *et al*, 2008). -Increase mildew disease (Russell *et al*, 2008). -Higher dark respiration rate (Okamoto, 1969). - leaves and shoot death. (Leigh and Wyn Jones, 1984) -Decrease root length. (Drew, 1975)	

In previous work Ridout and Thomas (2001) noted historical reports that Chevalier barley grows well under low nitrogen levels. In this study, modern and historic barley varieties were characterised to determine whether they differed in major agronomic features.

4.1.2 Aims.

The aims of the current study was to determine the effect of nitrogen addition on the growth of historic and modern barley varieties, to evaluate grain characteristics and to investigate the effect of nitrogen application on powdery mildew and FHB incidence.

4.2 Methods and experimental procedures.

To test the hypothesis that historic and modern varieties are similar, the effect of different levels of applied nitrogen on leaf and tiller number, extension and final leaf length and total plant development was determined for Chevalier barley (as historic barley cultivar) and compared with two modern barley cultivars (Tipple and Westminster) as detailed in experiment N1. To determine the effect of different levels of nitrogen on the yield of grain and plant growth features at maturity, a more extensive comparison was made with the same modern varieties and three historic varieties as detailed in experiment N2. To investigate whether there is an interaction between nitrogen level and the incidence of powdery mildew and FHB diseases, a more extensive comparison was made with the same modern varieties and five historic varieties as detailed in experiment N3.

4.2.1 Nutrient solution composition and treatments for nitrogen experiments.

Five nitrogen treatments 0.5, 1, 2.5, 5, and 10 mM KNO_3 were added to a basal nutrient solution containing K_2HPO_4 (0.3 mM), KH_2PO_4 (3.0 mM), $MgSO_4.7H_2O$ (2.4 mM), $CaCl_2.2H_2O$ (3.0 mM), $FeCl_3$ (5 mM) and micronutrients ($MnSo_4$ 0.151g, $ZnSO_4.7H_2O$ 0.029g, H_3BO_3 0.309g, $CoSO_4.7H_2O$ 0.02g, $CuSO_4.5H_2O$ 0.025g, NaCl 0.585g and $Na_2MoO_4.2H_2O$ 0.12g /L) were prepared as required.

The five nitrogen treatments (0.5, 1, 2.5, 5, and 10 mM KNO_3) were used to water each pot with a specific volume of 100 ml per pot from the initial germination stage until harvest date. The maximum dosage of 10 mM KNO_3 provides an equivalent of 234 Kg ha^{-1} over a 92 day growth period and is comparable to just over the maximum recommended field addition to ensure adequate crop production

(HGCA, 2005).

The rooting medium of all barley cultivars were flushed three times weekly with the appropriate nutrient solution.

4.2.2 Effect of nitrogen levels on barley growth – Nitrogen experiment 1 (N1), 2010.

Glasshouse trials were conducted initially in nitrogen experiment 1 (N1) using three spring barley cultivars (Chevalier as historic variety and Tipple and Westminster as modern varieties) to determine growth responses before ear emergence. Plants were grown under different levels of nitrogen as detailed in 4.2.1 between 20th April and 5th June 2010 for 45 days. Four seeds of each barley cultivar were planted in plastic pots containing a litre of sand with four replicates for each level of nitrogen. Plants were thinned to two plants per pot to replicate field densities.

Plants were harvested and divided into roots, stems and leaves. All plant parts were dried at 60°C to constant weight. Measurements were taken of the number and length of leaves, the number of tillers and the dry weight of leaves, stems, roots and total plant dry weight. Individual leaf length was taken by measuring the length between the leaf tip and the ligule of the leaf. Leaves were considered fully extended when ligules formed.

Further analysis on barley dry leaves were also conducted for CHN analysis by Chemispec as detailed 2.7.

4.2.3 Effect of nitrogen levels on barley growth – Nitrogen experiment 2 (N2), 2010.

A more extensive comparison between varieties was conducted in nitrogen experiment 2 (N2) between 20th April and 2nd September. Five varieties (Armelle, Chevalier and Oderbrucker as historic varieties and Tipple and Westminster as modern varieties) were planted and grown under different levels of nitrogen and

under the same conditions as in experiment N1 but with plants harvested at maturity for analysis of grain yield and for nitrogen content in seeds.

The same nitrogen treatments were given as described in 4.2.1. Plants were harvested at maturity for analysis of plant height, number of tillers, dry weight, grain yield and nitrogen content in seeds. To determine the effect of nitrogen level on malting barley quality, seeds of each pot in two varieties Chevalier and Tipple were ground separately and samples (20-50mg) analysed for CHN analysis as detailed in 2.7.

4.2.4 Powdery mildew and FHB scoring in nitrogen experiment 3 (N3) under glasshouse conditions, 2010.

Because application of nitrogen may affect the level of FHB disease and mycotoxin accumulation in cereal grains, the effect of nitrogen application on FHB disease was tested in seven barley varieties (Armelle, Chevalier, Oderbrucker, Plumage, and Vellavia as historic varieties and Tipple and Westminster as modern varieties). Plants were grown in sand under glasshouse conditions and with different levels of N concentrations as detailed in 4.2.1 between 20th April and 2th September. These experiments were conducted to determine the effect of N level on FHB and mildew (natural infection).

Seeds of each barley cultivar were planted in pots as detailed in 4.2.2 (N1). The design of the experiments was factorial with different barley cultivars, different N levels and different treatments (infected and uninfected plants) arranged as a randomized complete block.

Plants were infected with *F. culmorum Fu 42* as detailed in 2.5.2. All infected plants cultured under different nitrogen levels were scored for FHB and for naturally developing mildew disease as described in 2.4.1 and 2.4.2.

For the control treatment, five varieties were grown in a separate glasshouse as detailed in 4.2.3 (N2). Control plants of Plumage and Vellavia were not included due to seed shortage.

Plants were harvested at maturity for analysis of plant height, number of tillers, dry weight and grain yield.

4.2.5 Statistical analysis.

The design of all experiments with all barley cultivars was factorial (different barley cultivars and five nitrogen treatments, or barley cultivars, five N levels and infected or uninfected with FHB). Four replicates for each nitrogen concentration were arranged in randomized complete block. All data were checked for normality of distribution before analysis. Equality of variances was checked using Levenes tests. Where raw data was not normally distributed or the Levenes is significant (< .05), the data was transformed to log values. Other ways were also used to transform the data such as angular, Logit, square root, cube root, to the power $(x)^{1/4}$, to the power $(x)^{1/8}$, to the power $(x)^{1/3}$, reciprocal (1/x), cosine and sine.

The significance of the treatment for the nitrogen effects was determined by analysis of variance which was carried out on all data by using two-way and three-way parametric ANOVA within SPSS. When transformations were unsuccessful, one-way non-parametric ANOVA (Kruskal Wallis) tests were used to test if there is a significant difference between groups.

4.3 Results.

4.3.1 Effect of nitrogen levels on barley growth, Nitrogen Experiment 1(N1).

4.3.1.1 Effect of nitrogen levels on leaf number, tiller number and leaf extension.

Analysis for the main effect of nitrogen on leaf number, tiller number and leaf extension for all three barley cultivars is shown in Table 4.2. An increase in leaf and tiller number with increasing levels of the applied nitrogen is evident for all varieties. Treatments containing 5 or 10 mM produced a ≥ 2-3 and a ≥ 2-5 fold increase in leaf and tiller number respectively as illustrated in Figure 4.1 for Tipple.

Figure 4.1: Effect of nitrogen levels
(from left to right 10, 5, 2.5, 1 and 0.5 mM) on the growth of Tipple barley.

Table 4.2: Effect of nitrogen levels on leaf number, tiller number and leaf extension of modern and historic barley cultivars forty five days after sowing.

	a- Mean leaf number					One-way ANOVA between N levels
Varieties	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	*P*
Chevalier	10	12	19.25	26	36.5	< .001
Tipple	9.5	14.25	18.25	18.25	31	< .001
Westminster	14	14	16.75	27	30.5	.002
	b- Mean tiller number					
Varieties	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	*P*
Chevalier	2	2	5	6.25	10.25	.001
Tipple	2	3.25	4.25	4.5	8.25	< .001
Westminster	4	3.25	3.75	7.25	8.75	.005
	c- Leaf extension (cm)					
Varieties	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	*P*
Chevalier	12.85	17.26	18.46	21.83	20.9	.007
Tipple	12.69	15.74	20.11	18.99	21.73	.003
Westminster	18.04	21.29	20.76	22.38	24.07	< .001

	Leaf number One-way -parametric ANOVA					Tiller number One-way -parametric ANOVA				
	P	df	F	CV	LSD	P	df	F	CV	LSD
V	. 600	2	.515	.43		.523	2	.656	.53	
N (Angular)	< .001	4	83.971		3.515	< .001	4	58.338		1.274

Leaf extension Two-way parametric ANOVA	P	df	F	CV	LSD
V	< .001	2	14.823	. 20	2.697
N	< .001	4	22.470		
V*N	.195	8	1.470		

ANOVA analysis of variance, P probability, df degree of freedom, CV coefficient of variation, LSD least significant difference, V between varieties and N between N levels

The results presented in Table 4.2 show that Westminster barley produces a greater number of leaves and tillers at 0.5 mM applied nitrogen and had a 2 fold greater number of tillers compared to Chevalier and Tipple. Statistical analysis revealed no significant differences between varieties but there were significant differences between N levels (F (4, 55) = 83.971 and 58.338 for leaf and tiller number respectively, P < .001). Plants grown under high levels of N produced

greater leaves and tiller numbers compared with plants grown under low levels of nitrogen.

Overall no constant or significant differences in leaf or tiller number were seen between barley cultivars overall although Westminster plants do appear more robust.

The effect of N on leaf features is confirmed from analysis of leaf length. In general, leaf length of Westminster barley again was the greatest in comparison with Chevalier and Tipple varieties for most nitrogen levels (Table 4.2c). Statistical analysis using two-way parametric ANOVA revealed that there was no significant interaction between the effects of variety and nitrogen level on leaf extension (F (8, 45) = 1.470, P = .195). However, there was a significant difference between barley varieties and also between N levels (F (2, 45) = 14.823, F (4, 45) = 22.470) for varieties and N levels respectively (P = < .001). Westminster had significantly greater length of leaves particularly at low levels of N (0.5-1 mM).

4.3.1.2 Effect of nitrogen levels on leaf, stem and root dry weight.

Table 4.3 illustrates the changes of barley dry weight as a result of increasing the nitrogen level from 0.5 to 10 mM. The effect of increasing nitrogen levels caused increases in dry weight of all tissues.

Leaf and stem dry weight increased substantially with increasing applied nitrogen from 0.5 to 10 mM. In terms of leaf dry weight, Westminster had a significantly higher leaf dry weight compared with Chevalier and Tipple varieties particularly at nitrogen levels 0.5, 1 and 5 mM (Table 4.3a).

Stem dry weight increased gradually with increasing applied nitrogen level from 0.5 to 10 mM for all cultivars and Westminster again showed the highest mass particularly at low level of nitrogen addition (0.5 mM). However, there were no significant differences between the three varieties at higher levels of nitrogen (Table 4.3b).

Root dry weight in Chevalier and Westminster showed a strong increase when the applied nitrogen increased from 2.5 to 5 mM whereas at 10 mM the root mass was lower. At low levels of N, again the results showed that Westminster had a greater root dry weight mass compared with Chevalier and Tipple varieties (Table 4.3c).

Total plant dry weight increased progressively as applied nitrogen level increased from 0.5 to 5 mM (Table 4.3d). In general total plant dry weight was higher in Westminster at low levels of nitrogen (0.5 and 1 mM) compared to Chevalier and Tipple barley cultivars while dry weight was higher in Chevalier at high levels of nitrogen (2.5, 5.0 and 10 m^{-3}).

Statistical analysis using two-way ANOVA revealed that the interaction between varieties and N levels significantly affect all measures of barley growth as measured by dry weight of tissues (F (8, 45) = 7.064, 2.835, 4.078 and 4.768). The significant interaction shows that the effect of varieties is not consistent but the effect of N levels is consistent. For example, Westminster had greater total dry weight at low level of N while Chevalier was more productive at high levels of nitrogen (Table 4.3). This indicated that the combined factors of variety and N level had an effect on the dry weight of all tissues for leaf, stem, root and total dry weights respectively ($P < .05$). The main effects of varieties growing with different levels of nitrogen resulted in different dry weight of tissues. There was also a significant difference between varieties and between N levels for the total dry weight of tissues ($P < .05$).

Plants grown under high levels of N had significantly higher masses of weight compared to plants grown under low levels of N (Table 4.3).

Table 4.3: Effect of nitrogen levels on: a- leaf dry weight, b- stem dry weight, c- root dry weight and d- total dry weight per plant of barley (45 days after sowing).

a- Leaf dry weight Varieties (2 or 6 row)	0.5 mM	1 mM	2.5 mM	5 mM	10 mM
Chevalier	0.07	0.14	0.27	0.42	0.53
Tipple	0.05	0.15	0.25	0.27	0.47
Westminster	0.17	0.21	0.26	0.49	0.58
b- Stem dry weight (g)	0.5 mM	1 mM	2.5 mM	5 mM	10 mM
Chevalier	0.06	0.14	0. 21	0.37	0.42
Tipple	0.08	0.18	0.26	0.29	0.42
Westminster	0.15	0.20	0.23	0.40	0.42
c- Root dry weight (g)	0.5 mM	1 mM	2.5 mM	5 mM	10 mM
Chevalier	0.08	0.41	0.43	1.08	0.82
Tipple	0.09	0.09	0.15	0.20	0.43
Westminster	0.35	0.52	0.30	0.85	0.60
d- Total plant dry weight (g)	0.5 mM	1 mM	2.5 mM	5 mM	10 mM
Chevalier	0.21	0.69	0.91	1.86	1.77
Tipple	0.21	0.42	0.66	0.76	1.31
Westminster	0.66	0.92	0.79	1.75	1.60

Two-way parametric ANOVA

	Leaf dry weight (Log)					Stem dry weight (SQRT)				
	P	df	F	LSD	CV	*P*	df	F	LSD	CV
V	< .001	2	30.818	.110	.60	.002	2	7.385	.117	.52
N	< .001	4	168.744			<.001	4	108.113		
V*N	<.001	8	7.064			.012	8	2.835		
	Root dry weight					**Total dry weight (Log)**				
	P	df	F	LSD	CV	*P*	df	F	LSD	CV
V	< .001	2	24.804	.275	.84	< .001	2	22.205	.343	.62
N	< .001	4	18.826			< .001	4	59.237		
V*N	.001	8	4.078			< .001	8	4.768		

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference, CV coefficient of variation, V between varieties, N between N levels and V*N interaction between varieties and N levels.

4.3.1.3 Effect of nitrogen levels on nitrogen content of barley leaves.

In general, the high levels of nitrogen addition showed a higher nitrogen leaf content for all varieties (Table 4.4). Statistical analysis revealed no significant difference between the three barley varieties ($P = .052$) but there were significant differences between N levels ($P < .001$). The N content in leaves was higher for

plants grown under high levels of N compared to plants grown under low levels of nitrogen.

Table 4.4: Effect of nitrogen levels on the percentage of nitrogen content of leaves in barley varieties at harvest (45 days after sowing).

Variety	Percentage of nitrogen content in leaves					One-way ANOVA between N levels
	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	*P*
Chevalier	3.24	2.33	4.16	3.88	4.98	< .001
Tipple	2.52	4.37	4.25	4.76	5.63	< .001
Westminster	3.75	3.5	3.97	4.24	5.24	< .001

One-way parametric ANOVA				
	P	df	F	CV
V	.052	2	3.054	.24
One-way non-parametric ANOVA				
	P	df	Chi-square	
N	< .001	4	54.604	

ANOVA analysis of variance, P probability, df degree of freedom, CV coefficient of variation, V between varieties and N between N levels

4.3.2 Effect of nitrogen levels on barley at harvest, Nitrogen Experiment 2 (N2).

Glasshouse experiment 2 allowed for an analysis of the effects of nitrogen on the grain yield of plants and on the nitrogen levels in seeds. Five varieties were compared, Armelle and Chevalier historic two-row, Oderbrucker historic six- row and Tipple and Westminster modern two-row.

4.3.2.1: Effect of nitrogen levels on barley grain yield.

Barley grain yield increased significantly with increasing applied nitrogen levels from 0.5 to 2.5 mM (Table 4.5). Further increases in the applied nitrogen levels to 5 and 10 mM had either little or no effect on grain yield and in a number of cases reduced yield.

Statistical analysis revealed significant differences between varieties (F (4, 93) = 16.243), P <.001) and also between N levels (F (4, 94) = 10.570, P < .001). Plants grown under low levels of N produced lower grain yield compared to plants grown under higher levels of N.

In general, modern varieties Westminster and Tipple and Chevalier historic barley variety produced greater grain yield compared to historic varieties Armelle and Oderbrucker (Table 4.5).

Table 4.5: Effect of different levels of nitrogen on grain yield (g per plant).

Variety (2 or 6 row)	Nitrogen levels					One-way ANOVA between N levels
	0.5 mM	1 mM	2.5 mM	5 mM	10mM	*P*
Armelle (2)	0.80	1.48	3.05	2.73	1.94	< .001
Chevalier (2)	1.58	2.45	5.68	5	2.6	< .001
Oderbrucker (6)	0.36	0.66	1.9	2.85	2.7	< .001
Tipple (2)	**2.22**	**3.37**	**6.03**	**5.6**	**5.9**	**.013**
Westminster (2)	**3.11**	**4.28**	**5.55**	**6.85**	**5.88**	**.018**

One-way parametric ANOVA					
	P	df	F	LSD	CV
V (Logit)	< .001	4	16.243	1.646	.60
N	< .001	4	10.570	1.822	

ANOVA analysis of variance, P probability, df degree of freedom, CV coefficient of variation, LSD least significant difference, V between varieties and N between N levels

4.3.2.2 Effect of nitrogen levels on nitrogen content of seeds in barley.

Sample seeds from different nitrogen treatments of two varieties, Chevalier and Tipple were assessed for nitrogen content. The results indicated that nitrogen addition increased the nitrogen content in seeds of both varieties. Chevalier barley showed higher nitrogen content in seeds compared to Tipple seeds at all levels of nitrogen addition (Table 4.6).

Statistical analysis revealed significant differences between the two varieties (F (1,

58) = 7.609, P = .008) and also between N levels (F (4, 55) = 37.367, P < .001). The N content in seeds was higher for plants grown under high levels of N compared to plants grown under low levels of N.

Table 4.6: Effect of nitrogen levels on the nitrogen percentage of grains in historic and modern barley varieties.

Variety	Percentage nitrogen in seeds					One-way ANOVA Between N levels
	0.5 mM	1 mM	2.5	5 mM	10 mM	*P*
Chevalier	1.77	1.88	1.96	2.61	2.79	< .001
Tipple	1.26	1.34	1.81	2.2	2.58	< .001

One-way parametric ANOVA

	P	df	F	LSD	CV
V	.008	1	7.609	.848	.26
N (Angular)	< .001	4	37.367	.303	

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference, CV coefficient of variation, V between varieties and N between N levels

4.3.3 Effect of nitrogen level on powdery mildew disease incidence (N3).

The effect of applied nitrogen on the percentage of infected leaf area and number of infected leaves was investigated in 2010 as detailed in 2.4.1. At the first, second and third assessments at GS (16-38) mildew symptoms appeared with low levels in Chevalier, Oderbrucker and Armelle. However, the percentage of mildew infection was still higher in Chevalier barley compared to other barley cultivars as shown in appendix Table A3.

At the fourth assessment (GS 61-65), mildew infection levels increased with increasing applied nitrogen and the historic barley cultivars Chevalier, Oderbrucker, Vellavia and Armelle varieties were more susceptible to mildew compared to modern barley cultivars Tipple and Westminster. The results from seven barley varieties are detailed in Tables 4.7a.

The final assessment (GS 85-89) from the upper leaves showed that Chevalier, Oderbrucker and Vellavia were once again more susceptible to mildew compared

to modern barley cultivars (Table 4.7b).

Table 4.7: Effect of nitrogen levels on mildew disease a- % of infected area, b- % of infected leaves and c- % of infected plants on barley, glasshouse, 2010 (N3).

		a- GS 61-65 % infected area on all leaves				One-way ANOVA between N levels
Varieties (2 or 6 row)	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	*P*
Armelle (2)	0	0	1.8	15.33	23.43	.003
Chevalier (2)	38.29	34.05	51.58	48.22	43.17	.353
Oderbrucker (6)	19.44	26.94	26.73	45.32	37.50	.197
Plumage (2)	0	0	0	3	7.46	.001
Tipple (2)	**0.25**	**0**	**0.75**	**3.88**	**3.27**	**.069**
Vellavia (2)	15.63	10.38	18.27	24.88	46.80	.001
Westminster (2)	**0**	**0**	**0**	**0.25**	**0.83**	**1.00**
			% of infected leaves			
Varieties	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	*P*
Armelle (2)	0	0	18.54	54.57	51.84	.004
Chevalier (2)	61.92	55.98	60.00	61.94	58.83	.979
Oderbrucker (6)	23.96	63.54	37.42	46.17	38.25	.225
Plumage (2)	0	0	0	12.32	20.81	.001
Tipple (2)	**3.85**	**0**	**1.35**	**4.23**	**5.06**	**.185**
Vellavia (2)	29.84	31.33	35.13	20.93	26.57	.553
Westminster (2)	**0**	**0**	**0**	**0.90**	**2.12**	**1.00**
			% of infected plants.			
Varieties	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	
Armelle (2)	0	0	50	100	100	
Chevalier (2)	100	100	100	100	100	
Oderbrucker (6)	50	100	100	100	100	
Plumage (2)	0	0	0	100	100	
Tipple (2)	**33.33**	**0**	**33.33**	**100**	**100**	
Vellavia (2)	75	100	100	100	100	
Westminster (2)	**0**	**0**	**0**	**25**	**50**	

		b- GS 85-89 % of infected area from the three upper leaves				
Varieties	0.5 mM	1 mM	2.5 mM	5 mM	10 mM	*P*
Armelle (2)	0	0	0	0	0	
Chevalier (2)	18.38	30.38	24.13	30.38	48.75	.025
Oderbrucker (6)	39.13	38.75	13.75	37.5	43.75	.550
Plumage (2)	0	0	0	0	0	
Tipple (2)	**0**	**0**	**0**	**0**	**0**	
Vellavia (2)	53.13	32.5	48.75	60	65	.115
Westminster (2)	**0.38**	**0.5**	**0.63**	**0.75**	**0.25**	**.957**

Statistical analysis for Table 4.7 continued.

	% infected area One-way non-parametric ANOVA			GS 61-65	% infected leaves One-way non-parametric ANOVA			
	P	df	Chi-square	CV	P	df	chi-square	CV
V	< .001	6	108.073	1.12	< .001	6	98.389	1.02
	One- way parametric ANOVA				One-way parametric ANOVA			
	P	df	F	LSD	P	df	F	
N (Arcsine)	.014	4	3.240	8.832	.494	4	.853	

GS 85-89 One- way non-parametric ANOVA				
	P	df	Chi-square	CV
V	< .001	6	112.169	1.44
N	.903	4	1.041	

ANOVA analysis of variance, P probability, df degree of freedom, LSD least significant difference, CV coefficient of variation, V between varieties and N between N levels

Statistical analysis for the percentage of infected area revealed significant differences between varieties ($p < .001$). There were also significant differences between infection at different N levels for the fourth score (F (4, 150) = 3.240, P = .014) but there were no significant differences between infection at different N levels from the final score (P = .903). For the percentage of infected leaves, statistical analysis revealed significant differences between varieties ($P < .001$) but there were no significant differences between nitrogen levels (P = .494).

4.3.4 Effect of nitrogen levels on FHB disease incidence (N3), 2010.

The effect of nitrogen levels on FHB disease incidence was investigated under glasshouse conditions in nitrogen experiment 3 (N3). Barley plants grown with different levels of nitrogen were inoculated with *F. culmorum* or water as in control as detailed in 2.5.2. Infected heads were killed or damaged prior to grain fill and harvested grains showed characteristic visual symptoms of FHB including pink, chalky or pale grain colour.

A clear effect of nitrogen concentration is evident on the percentage of infected heads and grains by FHB for all varieties (Table 4.8). Statistical analysis revealed significant differences between varieties ($P < .05$), between infected and uninfected plants ($P < .001$) and also between different N levels ($P < .001$). The effect of N level revealed significant differences between infected barley varieties for the percentage of infected grains (F (4,180) = 51.529, $P < .001$).

In general, the levels of FHB infection differed considerably with Chevalier barley showing very limited infection compared to other varieties and with proportionately less infection for historic varieties at low nitrogen levels.

Overall, the disease incidence at harvest under glasshouse conditions was higher in modern barley cultivars (Tipple and Westminster) and historic variety (Vellavia) compared to Chevalier historic barley variety (Table 4.8).

Table 4.8: Incidence of *F. culmorum* infection on barley grown under different levels of nitrogen at harvest. a- percentage of infected grains and b- percentage of infected heads.

			a-Percentage of infected grains Varieties (2 or 6 row)						
N mM	Treatment		Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster **(2)**
0.5	Infected		2.78	1.19	13.20	29.17	**17.68**	1.56	**14.25**
	Control		25	0	0		**0**		**0.60**
1	Infected		17.35	1.80	8.34	49.77	**33.92**	23.33	**33.04**
	Control		3.10	0	0		**1.01**		**0**
2.5	Infected		24.23	17.87	16.85	16.34	**27.45**	45.58	**27.93**
	Control		1.70	0	0		**1.17**		**0**
5	Infected		75.68	28.16	54.60	33.56	**62.83**	62.69	**39.60**
	Control		18.59	4.88	0		**18.18**		**7.93**
10	Infected		71.37	64.87	86.41	76.75	**83.55**	78.40	**61.11**
	Control		67.58	11.23	8.33		**54.4**		**22.66**
One-way ANOVA	Infected	*P* =	.001	.001	< .001	< .001	< .001	< .001	< .001
between N levels	Control	*P* =	< .001	.003	.406		.036		1.00

			b- Percentage of infected heads						
N mM	Treatment		Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
0.5	Infected		25	14.29	50	75	**35.24**	12.5	**53.57**
	Control		25	0	0		**0**		**8.33**
1	Infected		58.33	21.43	50	100	**85.71**	70.83	**76.90**
	Control		37.5	0	0		**6.25**		**0**
2.5	Infected		91.67	30.36	58.33	100	**95.24**	100	**77.55**
	Control		8.33	0	0		**9.17**		**0**
5	Infected		73.75	81.67	73.33	77.5	**86.84**	82.5	**66.17**
	Control		58.33	19.05	0		**45.83**		**17.13**
10	Infected		93.75	78.57	87.5	83.75	**92.38**	86.61	**60.71**
	Control		87.50	37.92	8.33		**48.22**		**26.02**
One-way ANOVA	Infected	*P* =	.023	.002	.818	.185	.023	< .001	.308
between N levels	Control	*P* =	.053	.16	.406		.694		1.00

Statistical analysis for Table 4.8 continued.

		% Grain infection				% Head infection			
		One-way non-parametric ANOVA				One-way non-parametric ANOVA			
		P	df	Chi-square	CV	P	df	Chi-square	CV
Infected	V	.016	6	15.678	1.11	.003	6	20.115	.86
Control	V	.001	4	19.834		.001	4	19.065	
		One-way parametric ANOVA							
		P	df	F	LSD				
Infected	N	< .001	4	51.529	21.857	< .001	4	24.578	
		One-way non-parametric ANOVA							
		P	df	Chi-square					
Control	N	< .001	4	47.830		< .001	4	29.338	
	V	< .001	6	35.592		< .001	6	37.224	
	N	< .001	4	98.253		< .001	4	28.531	
	T	< .001	1	67.202		< .001	1	93.663	

ANOVA analysis of variance, P probability, df degree of freedom, CV coefficient of variation, LSD least significant difference, V varieties, T FHB treatment and N nitrogen level

4.3.5 Effect of nitrogen levels and diseases on barley growth (N3), 2010.

At harvest (GS 92), the effect of nitrogen levels and FHB disease on tiller number is evident. The effects of increasing applied nitrogen from 0.5 to 10 mM combined with FHB gave a ≥ 3-6 fold increase in tiller number (Table 4.9a). The results revealed that Oderbrucker, Plumage and Armelle varieties produced lower tiller numbers compared to other varieties under most levels of applied nitrogen.

In general, the results indicated that modern barley varieties Westminster and Tipple and historic variety Vellavia produce more tillers compared to other varieties. Moreover, there was no effect of FHB disease on number of tillers at low levels of nitrogen while at high levels of nitrogen FHB disease decreased the number of tillers especially in Chevalier, Tipple and Westminster (Table 4.9a). Statistical analysis revealed significant differences between varieties ($P < .001$) and also between N levels ($P < .001$). Plants grown under low levels of N produced lower tiller number compared to plants grown under high levels of N. However, there were no significant effect of FHB on tiller number ($P = .092$).

The effect of different levels of nitrogen combined with FHB disease on barley showed that there was no effect of nitrogen level and FHB on plant height at low levels of N however, at high levels of N most varieties were shorter (Table 4.9b).

Barley varieties Oderbrucker, Chevalier, Plumage and Vellavia are considered as taller varieties compared to Tipple, Westminster and Armelle confirming results in Table 4.9b. Statistical analysis revealed significant differences between varieties ($P < .001$) and also between N levels ($P < .001$). Plants grown under low levels of N produced shorter plants compared to plants grown under high levels of N. Nevertheless, there were no significant effect of FHB on plant height ($P = .120$).

Dry weight increased with increasing nitrogen level from 0.5 to 10 mM. Differences among varieties appeared to be related to plant height and number of tillers. The results indicated that there was no significant effect of FHB disease on dry weight at all levels of nitrogen ($P = .795$) (Table 4.9c). Statistical analysis for infected and uninfected plants revealed significant differences between varieties (F (6, 277) = 3.959, $P = .001$). However, there were no significant differences between varieties of infected plants ($P = .453$) nevertheless, there were significant differences between varieties of control plants ($P < .001$). There were also significant differences between N levels ($P < .001$). Plants grown under low levels of N produced lower mass compared to plants grown under high levels of N.

Overall, statistical analysis for all plant growth characteristic revealed significant differences between varieties and also between different levels of nitrogen but there were no significant differences between FHB infected plant and uninfected plants for all barley growth characteristics quantified in the harvested plants.

Table 4.9: Effect of nitrogen levels and FHB disease on plant growth of modern and historic barley cultivars at harvest. (Control plants for Plumage and Vellavia were omitted due to seed shortage).

a- Tiller Number

N mM	T	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
		Varieties (2 or 6 row)						
0.5	Infected	1.25	2.29	1.00	1.00	**3.29**	2.00	**2.71**
	Control	1.00	2.50	2.50		**2.75**		**3.50**
1	Infected	2.50	3.00	1.00	1.50	**3.86**	2.75	**4.00**
	Control	1.50	2.75	2.25		**3.50**		**5.25**
2.5	Infected	3.25	3.71	2.25	1.75	**6.29**	4.5	**6**
	Control	2.75	5.25	2.50		**7.25**		**8.5**
5	Infected	5.75	5.29	3.75	3.75	**8**	9.5	**11.14**
	Control	4.5	7.5	4.5		**11.75**		**14**
10	Infected	4.5	6.29	5.5	6	**8.29**	10	**13.57**
	Control	5	10.25	5.5		**20.25**		**19.75**
One-way ANOVA between N levels	Infected *P* =	.002	.005	.003	.004	.011	< .001	< .001
	Control *P* =	.002	< .001	.056		< .001		.002

b- Plant height (cm)

N mM	T	Armelle	Chevalier	Oderbrucker	Plumage	Tipple	Vellavia	Westminster
0.5	Infected	45.75	67.43	71.25	67.50	**44.86**	54.5	**52.71**
	Control	43.00	61.75	39.75		**43.5**		**58.5**
1	Infected	51.5	77.71	75	83.00	**56.29**	62	**56.43**
	Control	55.25	85.75	61.75		**58**		**57.5**
2.5	Infected	65	84.71	85.75	98	**62.71**	76	**71**
	Control	55.75	95.25	75		**62.5**		**68**
5	Infected	49.25	83.43	65.75	77.75	**61**	75.25	**61.00**
	Control	48.25	87.25	73		**63.25**		**66.5**
10	Infected	52.0	69.39	59.25	64.25	**56.86**	64.5	**57.57**
	Control	46	77.25	63.5		**58.25**		**59.25**
One-way ANOVA between N levels	Infected *P* =	.058	.023	.036	< .001	.021	.008	< .001
	Control *P* =	.015	.026	.181		< .001		.094

c- Dry weight

NmM	T	Armelle	Chevalier	Oderbrucker	Plumage	Tipple	Vellavia	Westminster
0.5	Infected	0.55	0.88	1.18	0.76	**1.29**	0.60	**1.01**
	Control	0.51	1.15	0.30		**0.78**		**1.50**
1	Infected	0.70	0.93	0.99	0.98	**2.27**	0.86	**1.33**
	Control	0.78	1.77	0.60		**1.74**		**2.23**
2.5	Infected	1.60	2.07	1.88	1.93	**3.54**	1.64	**2.37**
	Control	1.58	4.15	1.32		**3**		**2.90**
5	Infected	2.7	3.52	2.21	3.36	**4.39**	4.33	**4.88**
	Control	2.27	5.23	2.27		**3.66**		**5.03**
10	Infected	3.33	5.55	3.76	4.15	**5.3**	4.47	**5.69**
	Control	2.72	5.69	3.20		**6.46**		**6.17**
One-way ANOVA between N levels	Infected *P* =	< .001	< .001	< .001	< .001	.011	.005	< .001
	Control *P* =	< .001	< .001	< .001		< .001		< .001

Statistical analysis for Table 4.9 continued

		Tiller number					Plant height				
		One-way parametric ANOVA (Logit)					One-way non-parametric ANOVA				
		P	df	F	LSD	CV	P	df	Chi-square		CV
Infected (Logit)	V	< .001	6	7.312	3.043	.83	< .001	6	72.250		.24
		One-way non-parametric ANOVA									
		P	df	Chi-square							
Control	V	< .001	4	32.717			< .001	4	39.492		
		One-way parametric ANOVA					One-way parametric ANOVA				
		P	df	F	LSD		P	df	F	LSD	
Infected	N	< .001	4	34.145	3.158		< .001	4	11.653	13.722	
		One-way non- parametric ANOVA									
		P	df	Chi-square							
Control	N	< .001	4	49.800			< .001	4	6.754	15.157	
							One-way non-parametric ANOVA				
							P	df	Chi-square		
	V	< .001	6	62.176			< .001	6	112.117		
	N	< .001	4	132.768			< .001	4	52.168		
							One-way parametric ANOVA				
							P	df	F		
	T	.092	1	2.831			.120	1	2.438		

		Dry weight				
		One-way non-parametric ANOVA				
		P	df	Chi-square		CV
Infected	V	.453	6	5.734		.79
		One-way parametric ANOVA				
		P	df	F	LSD	
Control	V	< .001	4	7.420	1.761	
		One-way non-parametric ANOVA				
		P	df	Chi-square		
Infected	N	< .001	4	115.753		
		One-way parametric ANOVA				
		P	df	F	LSD	
Control	N	< .001	4	38.973	1.280	
	V	. 001 (Logit)	6	3.959	1.770	
	N	< .001 (Logit)	4	89.417	1.553	
	T	. 795	1	. 067		

ANOVA analysis of variance, P probability, df degree of freedom, LSD significant difference, CV coefficient of variation, V varieties, T FHB treatment and N nitrogen level

4.3.6 Effect of nitrogen levels and diseases on barley grain yield.

Barley grain yield increased with increasing nitrogen levels from 0.5 to 2.5 mM in infected and uninfected plants. Further increases in nitrogen levels to 5 and 10 mM had either little or no effect on barley grain yield. The grain yield decreased at high levels of nitrogen in both control and FHB treatments. However, in most varieties infected plants produced lower yields compared to the control especially at high levels of nitrogen. Statistical analysis revealed significant differences between varieties ($P < .001$), between infected and uninfected plants (F (1, 283) =

27.554, *P* = .001) and also between different levels of nitrogen (*F* (4, 279) = 17.793, *P* = .001). Statistical analysis for infected and control plants revealed significant between varieties and between N levels (*P* < .001). Plants grown under low levels of N produced less grain yield compared to plants grown under high levels of N.

Overall, modern barley varieties Westminster and Tipple produced greater yield compared to historic varieties as clearly shown in Table 4.10. A clear effect of the dose of nitrogen is evident for all varieties.

Table 4.10: Effect of different levels of nitrogen and FHB disease on barley yield.

		Barley grain yield (g)						
N mM	T	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
0.5	Infected	0.71	0.50	1.15	0.65	**1.40**	0.65	**1.80**
	Control	0.80	1.58	0.36		**2.22**		**3.11**
1	Infected	0.91	1.07	1.04	0.77	**3.47**	0.95	**2.18**
	Control	1.48	2.45	0.66		**3.37**		**4.28**
2.5	Infected	2.25	1.89	1.98	1.93	**4.65**	1.61	**3.36**
	Control	3.05	5.68	1.90		**6.03**		**5.55**
5	Infected	1.74	2.42	1.73	2.93	**3.33**	2.57	**4.24**
	Control	2.73	5	2.85		**5.60**		**6.85**
10	Infected	1.92	2.08	1.76	1.67	**2.76**	1.99	**3.87**
	Control	1.94	2.60	2.70		**5.90**		**5.88**
One-way ANOVA	Infected	*P* = < .001	.293	.093	< .001	.035	.001	.076
between N levels	Control	*P* = < .001	< .001	< .001		.013		.018

		One-way non-parametric ANOVA			
		P	df	Chi-square	CV
Infected	V	< .001	6	31.621	.74
		One-way parametric ANOVA			
		P	df	F	LSD
Control (Logit)	V	< .001	4	16.243	1.646
Infected	N	< .001	4	7.974	1.704
Control	N	< .001	4	10.570	1.822

	One-way non-parametric ANOVA			
	P	df	Chi-square	
V	< .001	6	59.544	
	One-way parametric ANOVA			
	P	df	F	LSD
N (Angular)	.001	4	17.793	1.845
T (Angular)	.001	1	27. 554	3.075

ANOVA analysis, P probability, df degree of freedom, CV coefficient of variation, LSD least significant difference, varieties, T FHB treatment and N nitrogen level

4.4 Discussion.

4.4.1 Effect of nitrogen levels on barley growth.

Different growth responses are suggested at different nitrogen levels whereby Westminster produced greater tiller and leaf number at low concentrations of nitrogen and Chevalier was greater tiller and leaf number at high concentrations (Table 4.2). The evidence presented here suggests that Chevalier can be equally productive as modern varieties of barley but requires different growth conditions as this variety is very susceptible to mildew disease under glasshouse conditions.

Under low levels of nitrogen barley plants had clear light green leaves. This is in agreement with Richards and Templeman (1936) who reported that light green leaves were associated with nitrogen deficiency. Statistical analysis indicates that the effects of different levels of applied nitrogen do not have the same outcome for all varieties (Tables 4.2). The results indicate that Westminster had better growth in comparison with Chevalier and Tipple at low levels of nitrogen.

The reason for the higher productivity in Westminster may result from this variety having a semi-dwarf gene (sdw1) on chromosome 3H (Ellis *et al*, 2002; White *et al*, 2009). This gene has an important role in reducing plant height and increasing productivity. Additionally, it also has effects on root characters e.g. root length and weight and also enhances the ability to absorb nitrogen and elements from the soil. Interestingly in this study greater root growth was evident in low nitrogen treatments for Westminster compared to Tipple and Chevalier (Table 4.3).

The greater tiller numbers found at high levels of applied nitrogen are likely to be due to the extensive development of secondary and higher order tillers. This is in agreement with Richards and Templeman (1936) who indicated that the tiller number, leaf number and individual leaf size reduced under nitrogen deficiency as a result of failure to synthesize acceptable amounts of protein associated with low meristematic activity.

The present results are in agreement with Spiertz and De Vos (1983) who

indicated the positive effect of nitrogen on increasing leaf biomass, leaf area, tiller number as well as greater grain yield production. Furthermore, Drew *et al* (1973) reported that high rates of applied nitrogen cause an increase in the number and extension rate of barley roots linked with increasing dry weight. The present results are also in agreement with Pearman *et al* (1977) who reported that vegetative growth and leaf area increases with increasing nitrogen application due to increases in respiratory loss of CO_2. They also revealed that the effect of nitrogen on vegetative growth and leaf area is greater compared to the effect on grain yield. This is supported by comparison of the yield data in Tables 4.5 and 4.10.

The rate of leaf extension was found to be greater at high levels of applied nitrogen in comparison with low levels of applied nitrogen. The reason for these results suggested by Andrews *et al* (1991) could be attributed to an increase in the number of cells and/or greater expansion of cells transversely. The present results are also in agreement with Metivier and Dale (1977) who indicated the effect of nitrogen on leaf extension and final length of barley plants.

Grain size could be also a factor for determining response to nitrogen level. Westminster which shows greater growth at low levels of applied nitrogen has a greater mean grain weight compared to Chevalier barley (1000 grains of Westminster barley weight of 49.17g compared to 47.07g for Chevalier barley) and there was significant difference between these weights (Table 3.14).

Overall, however, no clear or consistent difference is evident for growth characteristics between Chevalier as an example of historic barley varieties and Tipple and Westminster modern varieties. Chevalier at least may be considered comparable in growth and yield. The major issue of its height being unsuitable for mechanical harvesting is the most likely reason for its demise as a modern crop.

4.4.2 Effect of nitrogen levels on powdery mildew incidence.

Natural mildew infection was scored on all varieties and was more prevalent under high levels of nitrogen particularly on historic varieties Armelle, Chevalier, Vellavia

and Oderbrucker with 100% of Chevalier plants showing incidence at all levels of nitrogen. However, modern barley cultivars (Tipple and Westminster) as well as Plumage historic barley variety showed resistance against mildew disease even under high levels of nitrogen (5-10 mM) (Tables 4.7a and 4.7b).

Greater levels of mildew infection were recorded in most historic barley cultivars given high concentrations of nitrogen (5-10 mM). However, Chevalier barley showed high levels of mildew even under low levels of nitrogen (0.5 mM). In contrast, low levels of mildew (≤ 3.88) were recorded on modern barley cultivars (Westminster and Tipple) grown under both high and low nitrogen levels.

It has been found that the percentage of infected plants with mildew increased considerably at high levels of nitrogen (5-10 mM) even in Tipple and Plumage varieties. These results agree with Last (1962b) who reported that applying nitrogenous fertilizer increased mildew infection levels on Plumage barley.

Greater levels of mildew infection were noticed in plants exposed to high levels of nitrogen may result from the production of soft tissue with little resistance to penetration by fungal hyphae (Krauss, 1999). The present results agree with those of Russell *et al* (2008) and Jensen and Munk (1997) who indicated that higher nitrogen supply increased the development of mildew disease in barley by increasing the density of colonies and increasing number of spores per cm^2 leaf. Sander and Heitefuss (1998) also indicated that wheat plants with high nitrogen supplies had increased pustule numbers and more sporulation per unit leaf area compared to low disease intensity at low nitrogen levels.

4.4.3 Effect of nitrogen levels on FHB incidence.

Analysis of the percentage of infected grains and heads with FHB clearly showed that *F. culmorum* infection was more severe in all barley varieties grown under high levels of nitrogen compared to low levels of nitrogen (Tables 4.8). The incidence and the severity of FHB disease depended on the cultivar. For example, very high FHB levels were found in both Vellavia historic barley and Tipple modern barley while low levels were found in Chevalier. These results agree with Lemmens *et al*

(2004) who indicated that FHB disease severity and DON mycotoxin contamination increased with increasing applied nitrogen in wheat. Martin *et al* (1991) also reported that high soil nitrogen promoted FHB in cereals. Conversely, Fauzi and Paulitz (1994) and Lori *et al* (2009) indicated that there were no differences in FHB severity between regular and high fertilization levels under dry conditions. Nevertheless, the results reported here contrast with those of Yang *et al* (2010) whose results indicated that increased infection occurred in barley with low nitrogen levels and suggested that nitrogen fertilization is a possible way to minimise FHB in barley. The differences in these results may be related to the form of nitrogen as Huber and Watson (1974) indicated that the form of nitrogen available to plants may also affect the severity of disease. Comparative studies showed that applications of ammonium nitrate gave a higher level of FHB infected heads compared with the application of urea (Teich, 1987). The application of inorganic nitrogen in the studies reported here may reflect this.

The results of tiller number showed that the tiller number decreased under high levels of nitrogen. However, the results showed that there is no significant effect of FHB disease on tiller number overall (Table 4.9a). It has been found that greater tiller mortality occurred with barley cultivars which produced more tillers such as Chevalier, Westminster and Tipple. This condition is less likely in barley with less capacity to produce tillers (Armelle and Oderbrucker), and thus may be due to the competition between tillers for resources (Garcia del Morala and Garcia del Moral, 1995).

Plant height decreased slightly at high levels of nitrogen especially at the level 10 mM. The reason for these results may relate to the incidence of nitrogen being more in leaves, root and tiller growth. However, the results showed that there is no significant effect of FHB disease on plant height (Table 4.9b).

Dry weight increased with increasing levels of nitrogen. However, the results showed that there is no significant effect of FHB disease on plant dry weight (Table 4.9c). The differences between varieties may relate to the differences of plant height and number of tillers between varieties.

Increasing levels of nitrogen to 2.5 mM caused an increase of yield over all varieties possibly as a result of stronger productive tillering of plants and significant growth of leaves. This may be related to nitrogen effect on increasing biomass of leaf and stem tissue in addition to higher grain yield production (Birch and Long, 1990). However, further increases in nitrogen concentration to 5 and 10 mM had no or a negative effect on barley yield (Table 4.10). The results agree with Iles (2001) and Lauer and Partridge (1990) who found that increasing level of nitrogen has no clear effects on spring barley yield. A lower weight of grain yield of barley grown under high levels of nitrogen combined with a greater number of heads is related to decreases in grain size. This is in agreement with Lauer and Partridge (1990) who indicated that a high rate of N lead to increases in grain yield and protein, and decreases in grain size.

Overall FHB infected plants have less grain weight compared to plants grown under uninfected (control) conditions which may be related to a FHB disease effect in reducing grain yield via floret sterility as well as poor grain filling and grain shrinkage (tombstone grains) (Hatcher *et al*, 2003). However, the grain weight (g) reaches a maximum and then declines for most varieties indicating that too much nitrogen is sub-optimal. Overall, FHB severity can be minimized in different ways such as early planting date associated with suitable amount of N application (Subedi *et al*, 2007).

In summary, it appears that the incidence of mildew and FHB disease scores depended on the level of nitrogen provided. High levels of N were found to result in greater mildew and FHB incidence in both historic and modern varieties. Comparisons between barley varieties revealed that modern varieties are more resistant to mildew disease even under high levels of nitrogen (Table 4.7) while historic varieties are more resistant to FHB disease (Table 4.8). This may result from a number of causes including lower density of foliage and lower plant height or possibly due to the genetic causes, for example the presence of the mlo gene allele in modern varieties (HGCA Recommended List 2010).

Chapter Five

Physiology of infection and malting characteristics

5.1 Mechanisms and head characteristic in barley.

The results from Chapter Three presented evidence to suggest that Chevalier is more resistant to *Fusarium* infection compared to modern varieties Tipple and Westminster. The results also suggested a relationship between plant growth characteristics and resistance. To investigate this and to suggest possible mechanisms, further comparison of infection processes was judged to be valuable. In this Chapter, the characteristics of the grain are compared among the varieties to assess whether there are structural features that could account for resistance.

In wheat, there is a high level of resistance against FHB disease which has been identified in Chinese wheat cultivar Sumai 3. A major Quantitative Trait Locus (QTL) on chromosome 3BS and extra minor QTL have been detected in these cultivars which are employed in wheat-breeding programs (Buerstmayr *et al*, 2003; Liu and Anderson, 2003). However, in barley the sources of FHB resistance are limited, particularly in six-row varieties. Numerous QTL on chromosome 2H have been recognized for reducing FHB damage, DON content, and grain discoloration and may be exploited to enhance resistance against FHB in barley (Bai and Shaner, 2004). In general, the QTL for FHB resistance identified in barley indicates that the resistance in barley is partial. Genetic analysis conducted by Dahleen *et al* (2003), showed two FHB QTLs on chromosome 2H and one on chromosome 6H, which are also associated with low DON levels and a later heading-date.

Overall, the resistance of wheat against FHB disease in the field has indicated that genotypes with awns and dwarf genotypes are more susceptible under natural epidemic condition but not under artificial infection (Mesterhazy, 1995). On the other hand, Pekkarinen (2003) indicated that plant height, thickness and strong plant stem affect barley resistance against FHB. This may relate to shorter or lodging stems being easier to attack by soil-born spores which may reach the heads of shorter stems more easily.

A study conducted by Boddu *et al* (2006) indicated that the response of susceptible wheat and barley genotypes to FHB phenotypes is different. For example, the infection in extremely susceptible wheat spreads from floret to floret

and leads to infection of the whole head. However, in barley the infection is mostly limited to the first infected florets. Germination of *Fusarium* spores on barley takes a longer time (24h) after inoculation compared to on wheat (6 -12h) which possibly induces barley to counter the infection by limiting its spread. This postponement may be related to physiological, morphological or anatomical characteristics of barley flowers which differ from wheat.

Microscopic analysis conducted by Boddu *et al* (2006) revealed that infection with *F. graminearum* in barley occurs through three phases. The first phase from 0 to 48h after inoculation restricts the fungal progress and is associated with low DON accumulation. The second phase from 48 to 96h shows a greater fungal growth and active infection associated with increased DON accumulation. The final phase between 96 and 144h shows more growth of fungal hyphae and extensive DON accumulation.

The barley grain is composed of three major parts a grain coat, the endosperm and the embryo (Figure 5.1). The seed coat consists of three essentials: the husk, the pericarp and the testa. The husk layer which contains dead cells is organised as a honeycomb design and plays a role to protect the seed (Hornsey, 1999).

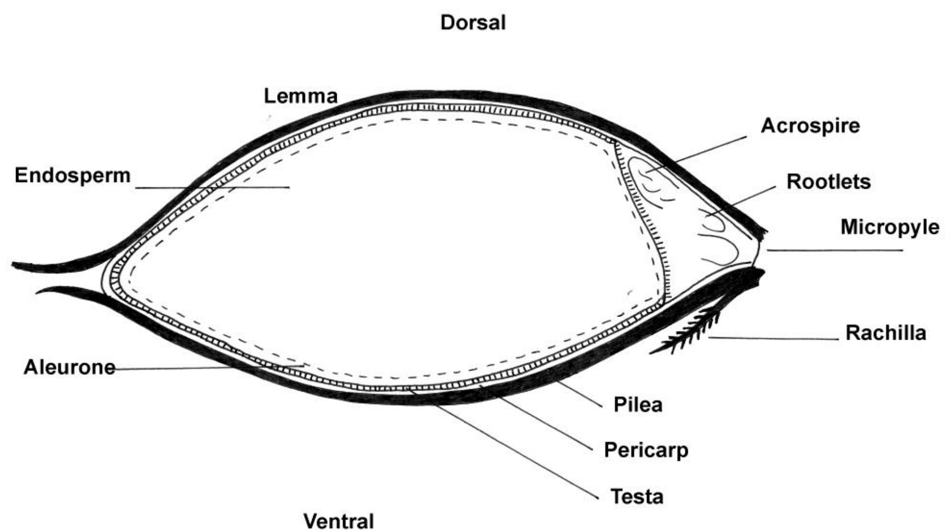


Figure 5.1: A longitudinal section of a barley seed showing the structure of barley seed: endosperm, seed coat (husk) and embryo.

A study conducted by Zhang *et al* (2008) showed that the infection pattern in both wheat and barley by *F. graminearum* is similar. The fungal spores germinate in infected spikelets on the top of the ovary or inner surface of the lemma and palea and grow toward the rachis of the next spikelets to infect the whole head. The difference between susceptible and resistance cultivars is that in resistant cultivars symptoms appear in individual spikelets at 6 days after inoculation and stop at rachilla. However, in susceptible cultivars the fungal growth extends towards the rachis and infects the neighbouring spikelets by extending growth upward and downward along the rachis. This study also reported that the symptoms of FHB appear in barley one day earlier compared to wheat. Another study using microscopic analysis conducted by Kang and Buchenauer (2000b) showed that the spores of *F. culmorum* take a longer time in resistant varieties to produce hyphae, which branch directly after germination on the head surface of wheat.

In barley, microscopic analysis conducted by Jansen *et al* (2005) revealed that successful penetration by *F. graminearum* destroys the fruit coat layers and eventually the fungus reaches starch and protein accumulating in the endosperm. After hyphae reach the rachis they continue to grow into apically located developing grains. Another study conducted by Skadsen and Hohn (2004) revealed that the fungus *F. graminearum* rapidly colonises the brush hairs of barley during the initial 7h followed by rapid basipetal growth alongside the pericarp epithelium (inner to the lemma and palea). Slower growth occurs inward through the pericarp and testa. Nevertheless, the aleurone and starchy endosperm may persist uninfected up to 16 days after infection. Furthermore, Lewandowski *et al* (2006) observed only occasional penetration of *F. graminearum* through barley stomata.

In general, penetration of the husk, pericarp and seed coat of barley by *Fusarium spp.* is related to its ability to produce enzymes with lytic activities against β-(1-4)-glucosidic and β-(l-4) xylosidic linkages particularly during the late fungal infection of the husk (Schwarz *et al*, 2002). For example, *F. culmorum*, *F. graminearum* and *F. poae* have the ability to produce alkaline proteinases as they grow in cereal protein media. An investigation conducted by Kikot *et al* (2010) revealed that some isolates of *F. graminearum* produce different extracellular enzyme activities

to degrade the essential components of the plant cell wall *in vitro*. The first enzyme produced is pectinase followed by hemicellulases and cellulases. This may be related to the necessity of pectic enzymes to increase the availability of cell-wall components for degradation by additional enzymes, cell lyses and plant tissue maceration. Similar results are reported in wheat and similar cell wall degrading enzymes are produced by *F. culmorum* including cellulases, xylanases and pectinases in infected heads during its colonization (Kang and Buchenauer, 2000a).

In addition to enzyme secretion, Kang and Buchenauer (1999) proposed that the hyphae of *F. culmorum* produce toxins during growth on the surface of the lemma, the ovary and parenchyma cells 36h post inoculation in wheat. These toxins increase with the progress of fungal growth especially in the cells in contact with fungal hyphae.

Various studies have revealed that fungal growth seems to be much slower in resistant wheat and barley varieties compared to susceptible ones (Zhang *et al,* 2008). The differences between resistant and susceptible wheat cultivars as indicated by Kang and Buchenauer (2000b) showed that in resistant cultivars the fungal growth is slower, the apposition layers are thicker and the papillae in infected tissues of the resistant cultivars are larger. In addition β -1, 3-glucan was noticed in the appositions and papillae and lignin accumulated intensely in cell walls. Lower accumulation of DON mycotoxin in infected heads has been found in resistant wheat cultivars compared to susceptible ones.

In general, barley is able to resist direct penetration of *F. graminearum* due to its florets external surfaces having thick-walled epidermal cells. However, the wall surfaces inside the floral cavity have thin-walled and susceptible cells. Lewandowski *et al* (2006) found that the fungus moves into florets frequently through crevices between the overlapping lemma and palea or through the top of florets possibly because the crevices are open for approximately 8 days after heads emerge. Fungal hyphae found on the external surface of florets in a protected pocket close to the base of the ventral furrow of the palea, extend to the crevice between lemma and palea. The testa or aleurone also possesses

obstacles against *F. graminearum*, such as proanthocyanidins and catechin as indicated by Skadhauge *et al* (1997). These may form a resistance mechanism and have been found in the testa of mature barley grains. *In vitro* assays by Carlson *et al* (2006) indicated that hordothionin, expressed as a transgene is an antifungal protein found in the barley endosperm and may be effective in decreasing *F. graminearum* growth. Boddu *et al* (2006) found that the interaction between barley and *F. graminearum* lead to induction of genes encoding defence-response proteins, oxidative burst-associated enzymes and phenylpropanoid enzymes. Furthermore, thionins, including the storage protein hordothionin belong to a class of small high-cysteine proteins found in barley are also useful as antimicrobial material against fungi (Nuutila *et al*, 1999).

A study conducted by Yoshida *et al* (2005) indicated that the effect of flowering type on the resistance of barley against FHB is higher than row type and other characters such as, wax coating, grain density, a semi-dwarf trait type and lateral floret size. For example, cleistogamous (non-opening self-pollinating flowers) varieties have low levels of FHB scores compared with chasmogamous varieties which are commonly susceptible. Mesfin *et al* (2003) indicated that lower FHB disease levels in two-row barley cultivars can be attributed to the unsuitable conditions for fungal growth such as more aeration and ventilation among the leaf canopy compared to six-row barley. However, in six-row barleys it was noticed that the fungus moves from one floret to another within the head without penetrating the rachis (Langevin *et al*, 2004).

Overall, resistance against FHB disease has been found more frequently in two-row barley and in varieties with purple lemma, long glume awns, taller plants and resistance to lodging and was not found in barleys with long rachilla hairs and rough lemma awn (Choo *et al*, 2004).

The barley varieties studied here show a range of physiological characteristics some of which may be instrumental in resistance to FHB. A comparison of those varieties showing different infection levels in field and glasshouse studies (Armelle, Chevalier, Oderbrucker, Plumage, Tipple, Vellavia and Westminster) may indicate features which could provide indications of mechanisms of resistance.

Observation of external colonisation, internal seed structure and internal colonisation are particularly relevant and were undertaken using light and electron microscopy.

While the effect of FHB infection on barley yield is one issue, the effect of FHB on malting efficiency of barley is a further concern and was investigated using seed from infected plants obtained from the 2009 preliminary trial. Malting is a complex process as outlined earlier. However, seed germination is critical and a high incidence of over 95% is required for commercial malting.

In general, the malting quality of a specific cultivar is determined by genetics, environment and malting practice (Li *et al*, 2008). For example, genotype and environmental conditions affect barley hardness and this feature is inherited which helps breeders grow very hard or soft varieties.

Adequate hydration of barley endosperm is very important to obtain good quality malt. Sub-optimal hydration of endosperm alters barley during malting which will be difficult to complete. It is also associated with poor friability scores (friability is the measure of a malt's readiness to crumble during milling and is important to determine and evaluate the general processing quality of malt) (Bryce *et al*, 2010). Assessment of grain response to water uptake is determined by incubating grains in different amounts of water to assess germination and water absorbance as indicators of malting performance.

The investigations reported here assess the physical characteristics of barley grains which could contribute to *Fusarium* resistance and germination and water absorbance as indicators of malting performance. This assessment is particularly focused on a comparison between modern varieties Tipple and Westminster and Chevalier as a historically renowned malting barley. If assessments indicate that Chevalier has comparable malting qualities then it will further substantiate the development of the variety for future breeding potential.

5.2 Materials and methods.

Hexamethyldisilazane (HMDS) for scanning electron microscopy investigation was purchased from Sigma Aldrich.

5.2.1 Light and electron microscopic observations.

In order to visualize the morphological characteristic of barley heads infected with *F. culmorum* and to view the pattern of the pathogen development in different barley varieties, observations using light and scanning electron microscope (SEM) were carried out at the University of Sunderland.

Barley heads of infected plants grown under infected glasshouse conditions, 2010 (G 2010) as detailed in 2.5.2 and 3.2.2.2 were harvested and prepared for SEM investigation. Samples comprising two heads and four individual seeds of Armelle, Chevalier, Oderbrucker, Plumage, Tipple, Vellavia and Westminster varieties were fixed and prepared for scanning electron microscopy (SEM). The samples were first fixed with 20% glutaraldehyde in PBS (phosphate-buffered saline) for 3 hours and washed twice with distilled water for 5 min. Glutaraldehyde fixed samples were passed through a graded ethanol series 50, 70, 90, 95, and 100% for 10, 15, 15, 2x20 and 3x20 min respectively. The samples were then infiltrated with HMDS through two incubations, firstly in 50:50 HMDS:ethanol for 30 min and the secondly in 100% HMDS for 30 min. At this point the samples were dried overnight in a fume hood and stored in a desiccator until used. The samples were splutter coated with gold in argon and examined by SEM.

To determine whether barley varieties differ in their response to *F. culmorum* infection during malting, further SEM investigations were conducted with intact barley seeds. Ten seeds of each variety sourced from the collection were immersed in a water suspension containing $5x10^5$ macroconidia ml^{-1} with 1 drop of tween 80. Infected barley grains of each variety (Armelle, Chevalier, Oderbrucker, Plumage, Tipple, Vellavia and Westminster) were placed on two wetted filter papers in the bottom of each petri dish and incubated at 23^oC. Five inoculated seeds were taken and fixed and prepared for SEM observation 3 days and another five seeds 7 days after incubation.

5.2.2 Germination energy of barley.

Barley grains of Chevalier, Tipple and Westminster harvested at Nafferton farm in 2009 (F 2009) after infection with *F. culmorum* as described in 2.5.2 were investigated for germinative energy (GE) as an indicator of malt quality.

The germination was carried out for four days in 90mm petri dishes for each variety. Germinated seeds counts were determined by placing 100 randomly selected barley grains of each sample on two filter papers in the bottom of each petri dish with ventral surfaces in contact with the paper in order to avoid drowning the embryo.

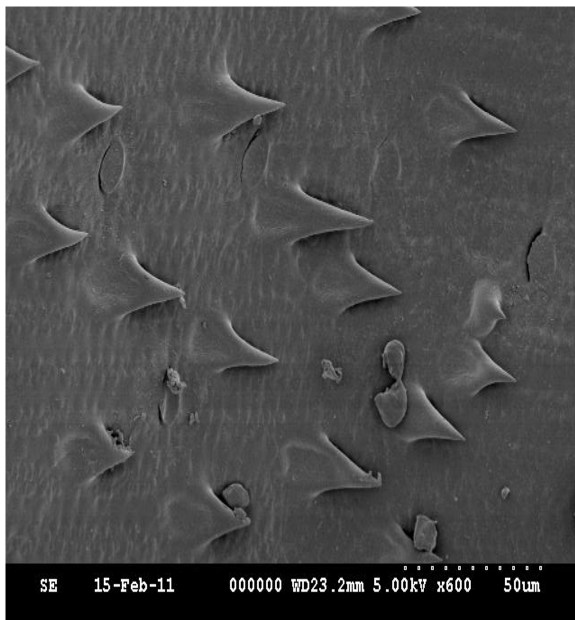
In order to compare differences between water uptakes between barley varieties, two amounts of sterile distilled water, 4 ml and 8 ml were used. All petri dishes were covered with lids and placed inside a plastic re-sealable bag to maintain constant humidity and incubated at 19.5±1.5^{o}C for 4 days.

The number of germinated grains was recorded at 24, 48, 72 and 96h from the beginning of steeping. Chitted grains were removed in order to avoid excessive moisture uptake by those seeds which germinated early. A seed was specified as germinated when the root was visible. The results were reported as a percentage of germinative energy on 4 ml and 8 ml water.

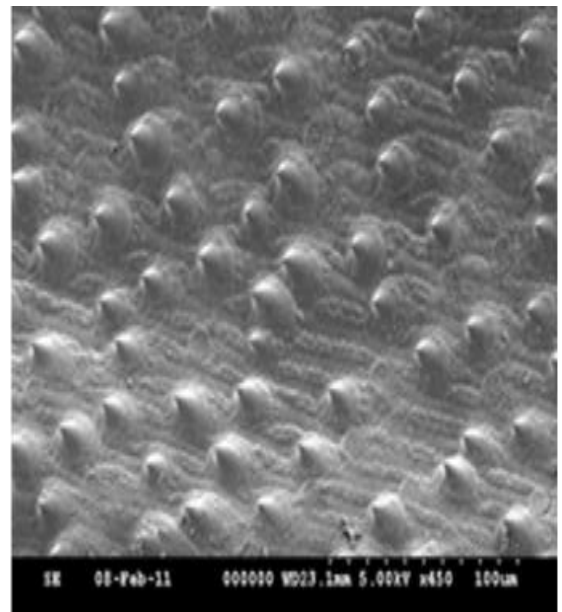
5.3 Results.

5.3.1 Grain characteristics.

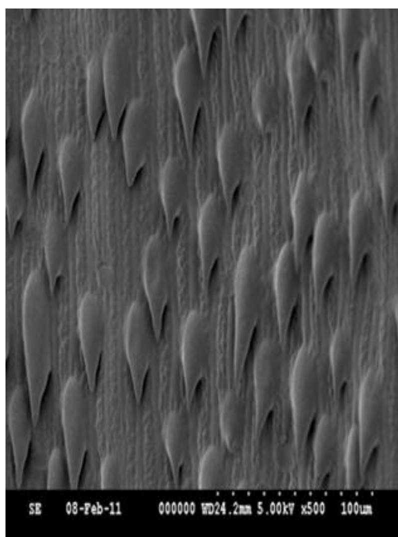
Scanning Electron Microscopy (SEM) showed that the outer surfaces of most two-row barley cultivars are similar with regular lined patterns across the grain. All varieties show raised pointed trichomes but Armelle and Oderbrucker have longer pointed trichomes which are clearly raised above the surface (Figure 5.2).



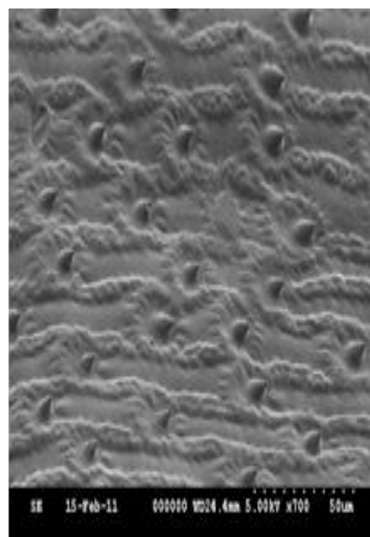
Armelle



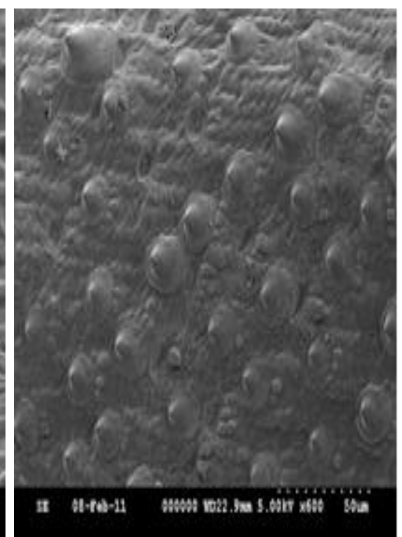
Chevalier



Oderbrucker



Plumage



Tipple

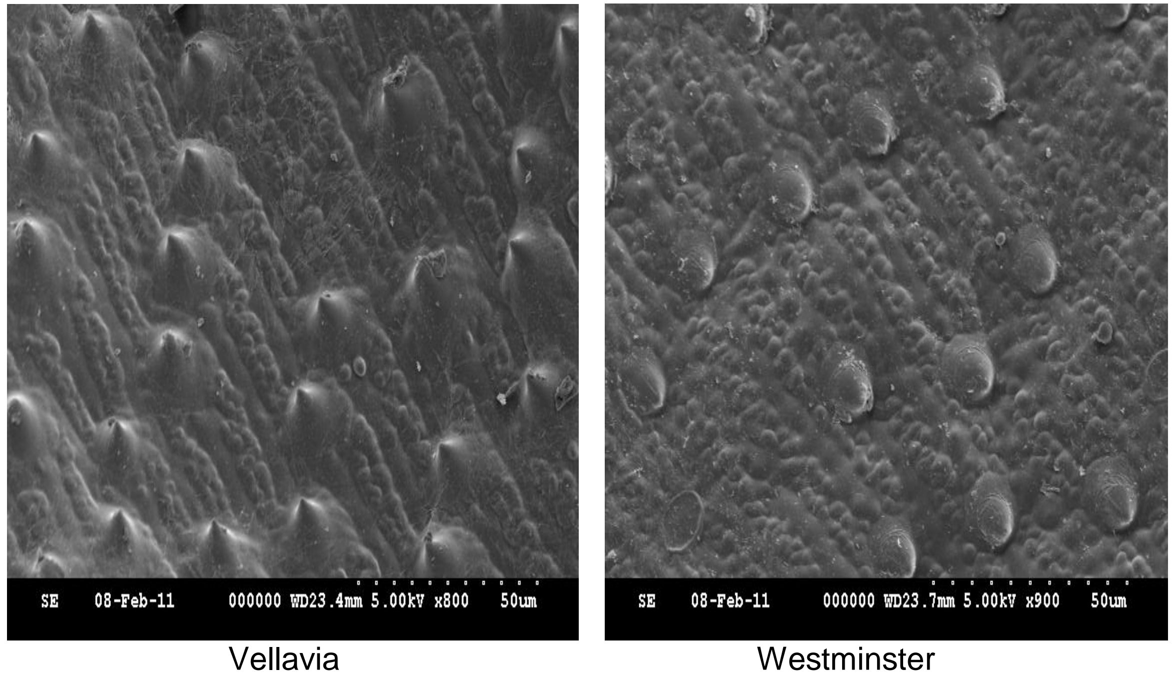
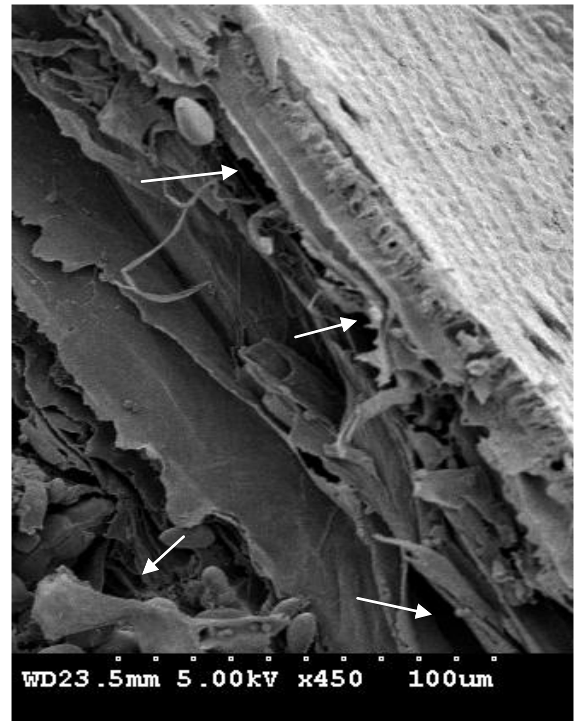
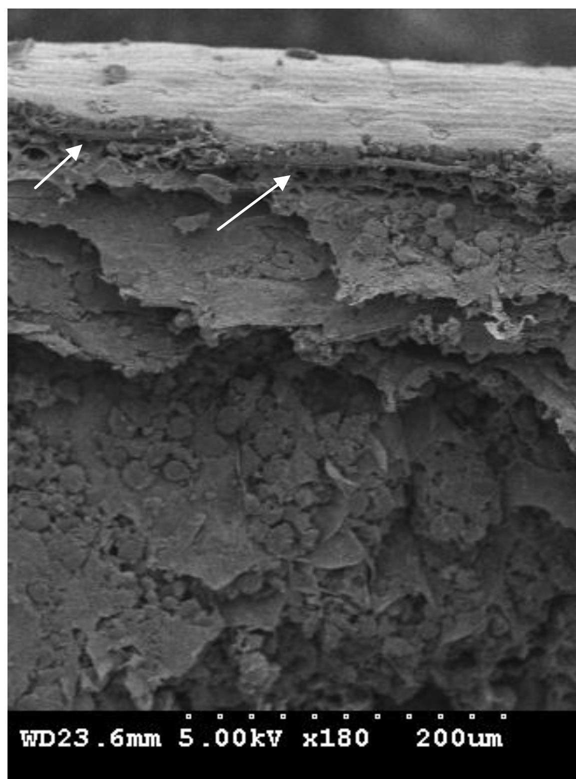


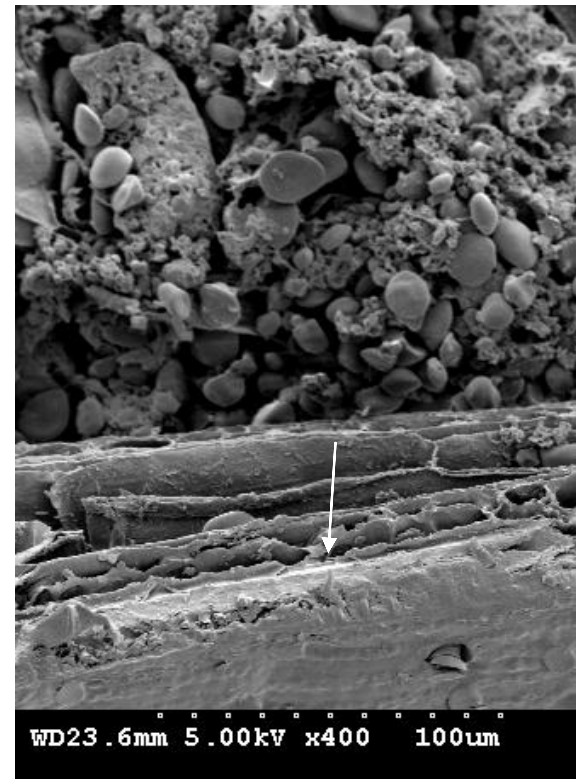
Figure 5.2: Outer surface and trichome features of barley grains showing the differences between different barley cultivars.

Scanning electron micrograph of longitudinal sections of grains cut through the ventral furrow showed that the lemma and palea differed between barley cultivars (Figure 5.3). Vellavia and Oderbrucker in particular were thinner and have a more open cellular or honeycomb structure than other varieties. A further difference between barley varieties was in the depth of the husk layers where some varieties such as Chevalier and Tipple show a limited depth between the lemma and the underlying endosperm but others such as Armelle and Plumage have more extensive spaces between the tissues.

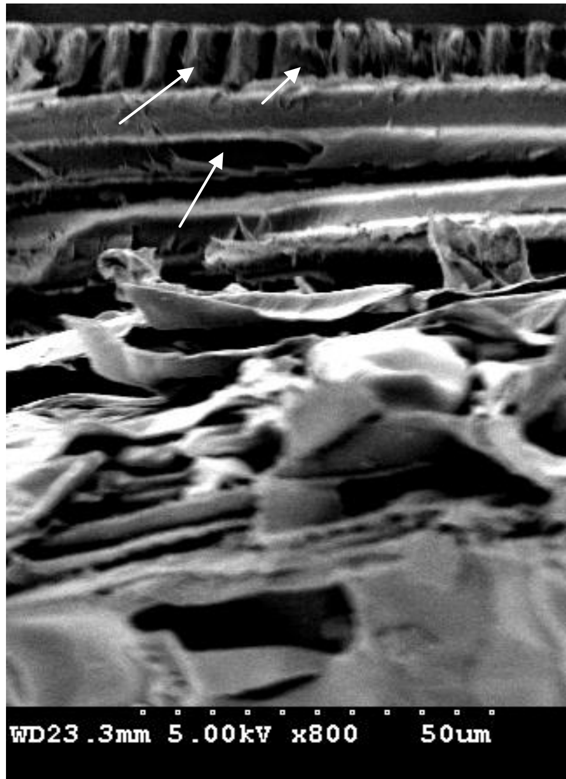


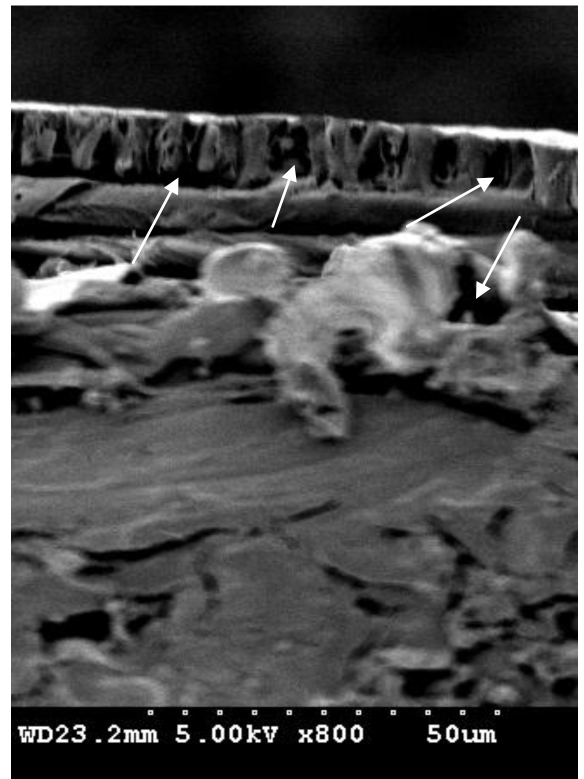
Armelle (Arrows show spaces between tissues)



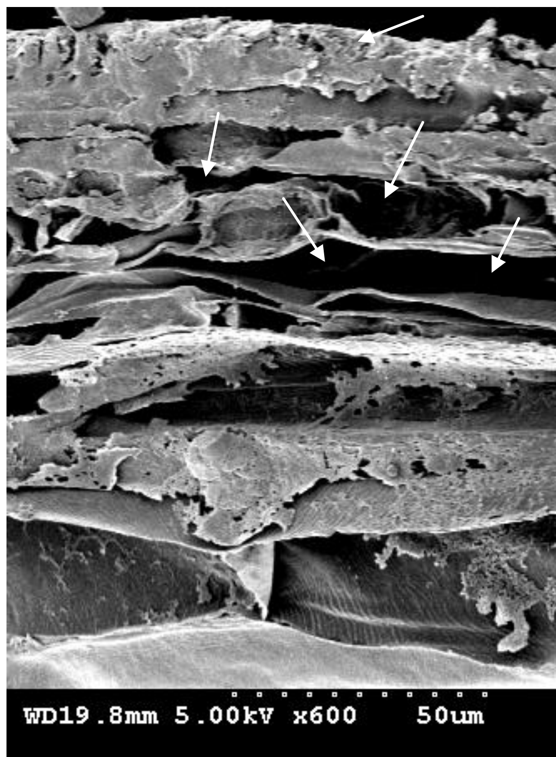


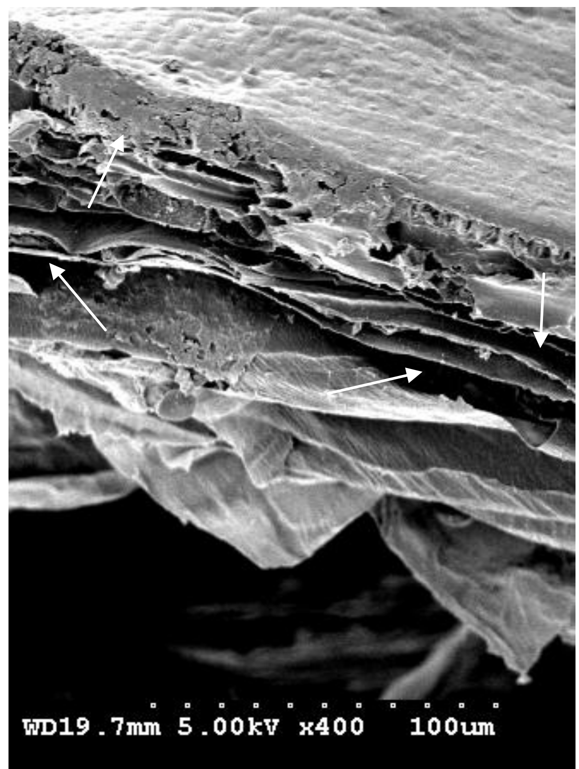
Chevalier (Arrows show limited depth of the husk layers)



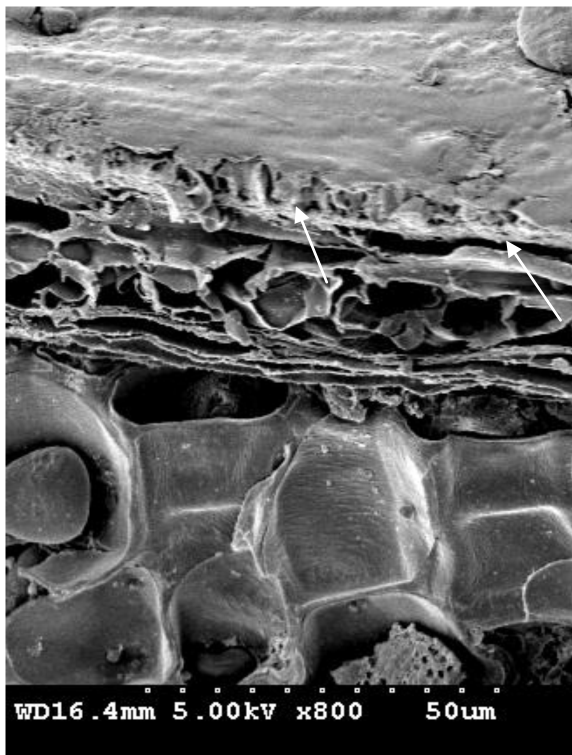


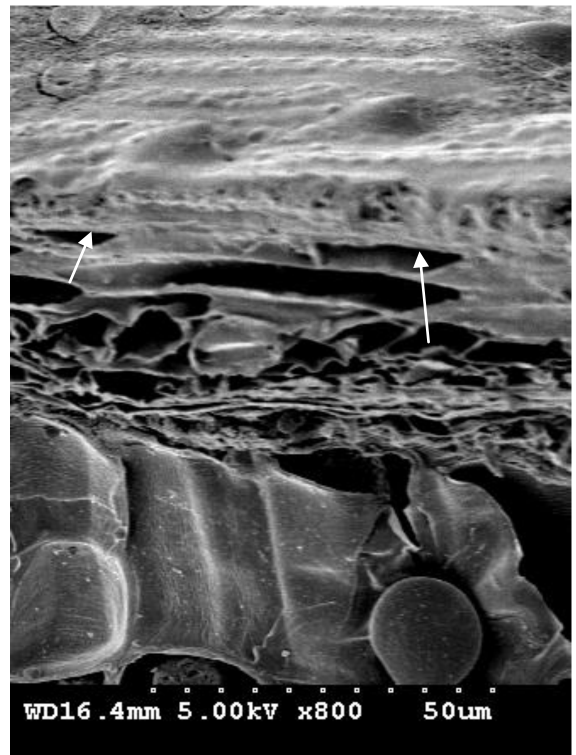
Oderbrucker (Arrows show limited depth of the husk and open honeycomb structure)



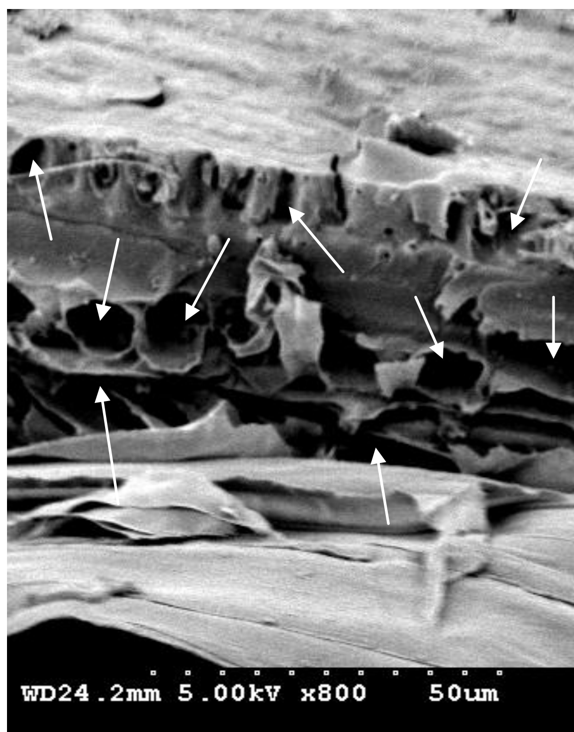


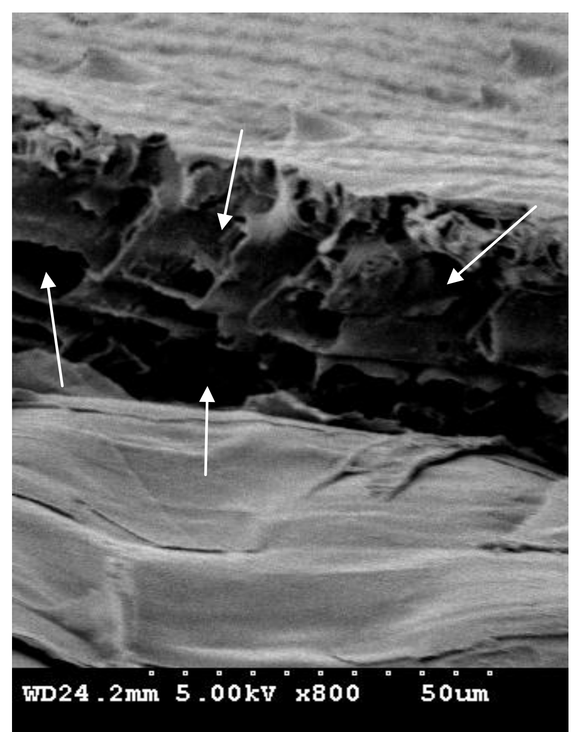
Plumage (Arrows show depth of the husk layers and spaces between tissues).



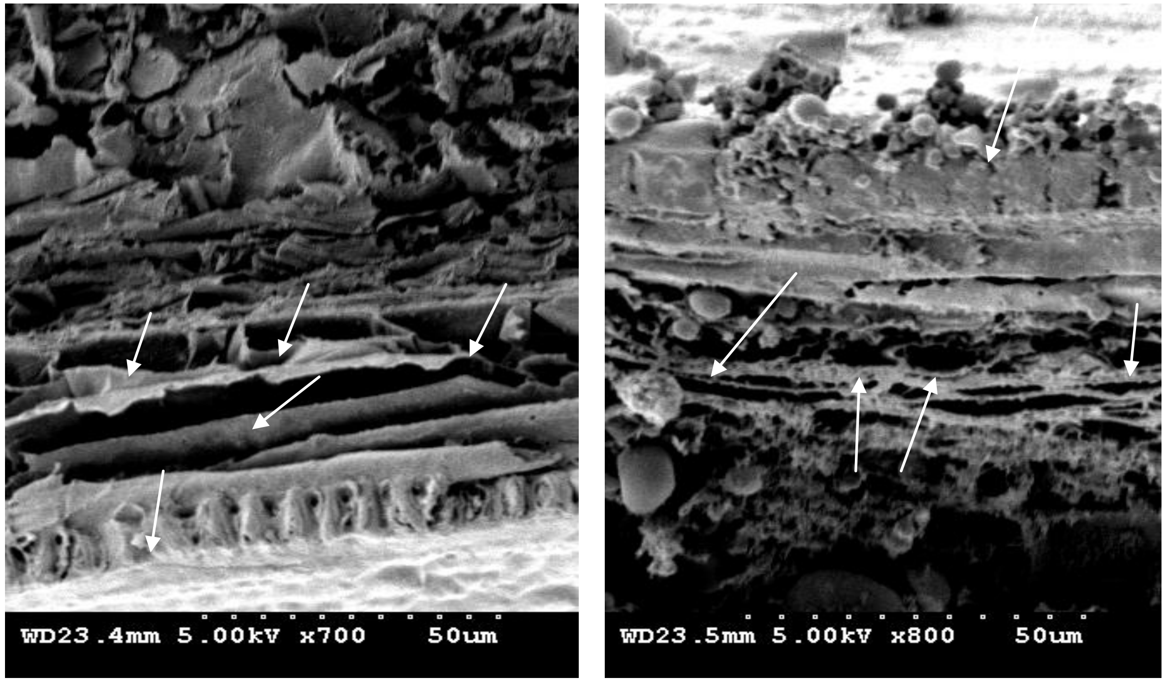


Tipple (Arrows show limited depth of the husk layers).





Vellavia (Arrows show open honeycomb structure and spaces between tissues)



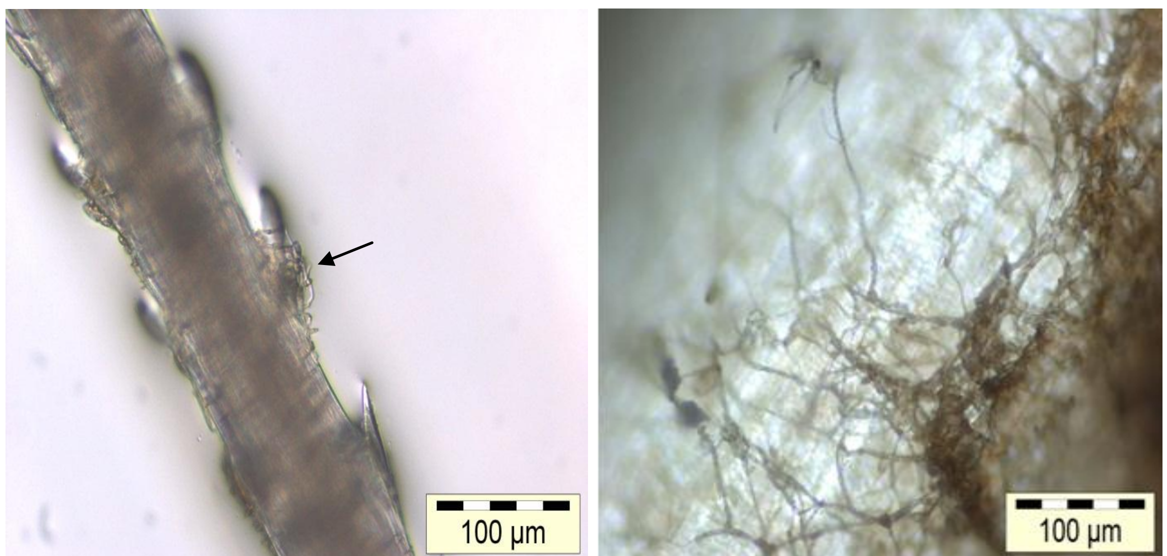
Westminster (Arrows show depth of the husk layers and spaces between tissues)

Figure 5.3: Longitudinal section of grains of barley cultivars showing husk features.

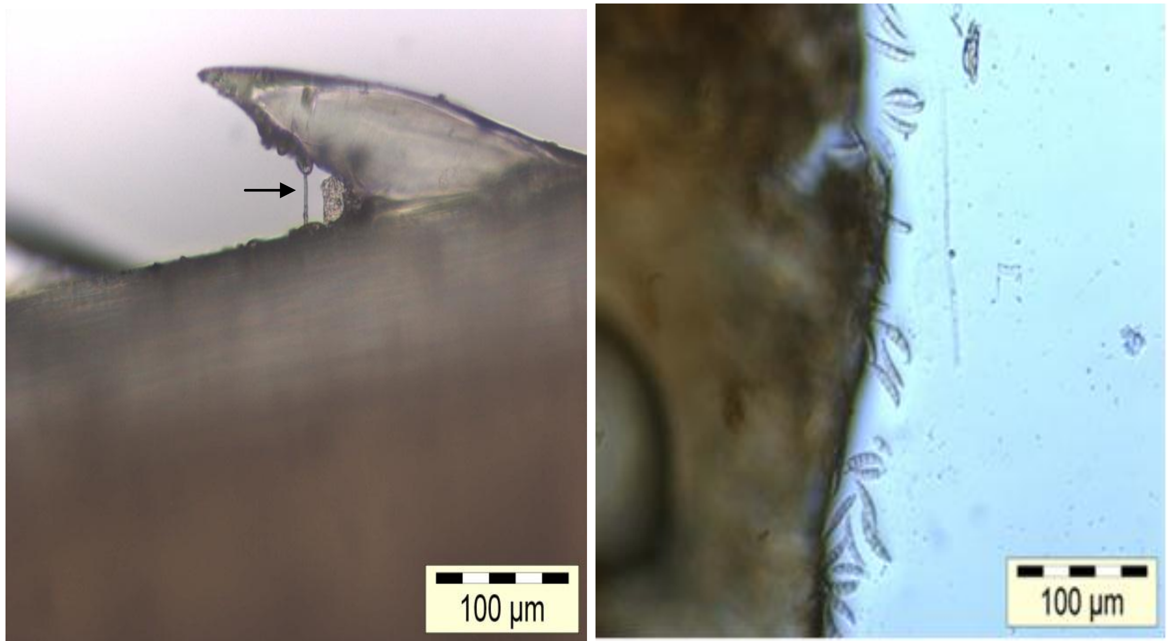
5.3.2 Fungal colonization.

5.3.2.1 Infected growing seed heads.

Light microscope observations showed macroconidia and fungal hyphae development on the heads awn surface as well as the outer surfaces of grains with differences among varieties (Figure 5.4).



Armelle (awn) Armelle (seed)



Oderbrucker (awn) Vellavia (awn)

Figure 5.4: cross-section of barley heads (Arrows show fungal hyphae growth and macroconidia (Vellavia).

From the outer surfaces of the inoculated barley heads, SEM investigation showed that the germinated macroconidia produced one to numerous germ tubes which grew and branched on the surfaces. However, the fungal development was less in Chevalier barley cultivar in contrast to other barley varieties.

In general, heavy fungal colonization was observed on the surface of Armelle grains and awns. Conidia were found to grow preferentially on trichomes near the seed tips and more hyphae and macroconidia as well as expanded colonies could be observed growing on the outer surface of the lemma extending to the outer surface of glumes and awns (Figure 5.5).

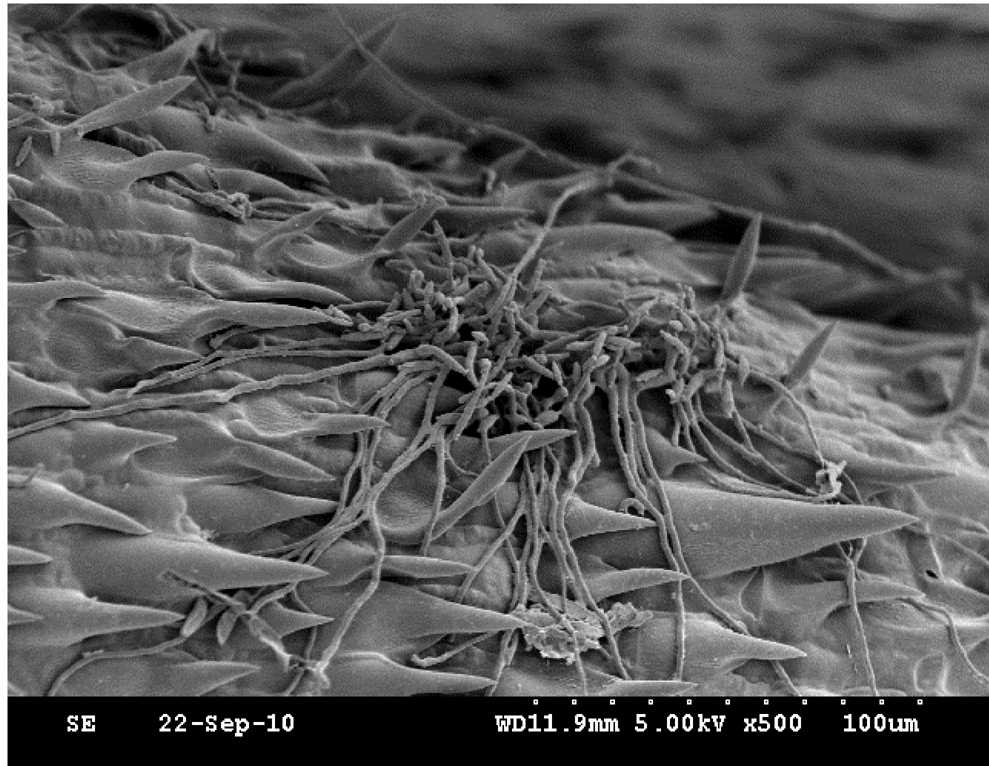


Figure 5.5: Colonisation of fungal growth on surfaces of Armelle barley seeds showing hyphal network between trichomes.

Furthermore, it was evident that some grains of Westminster barley were completely covered with a long, thick mycelium combined with macroconidia which is very clearly observed on the outer surface of the palea (Figure 5.6). However, in Chevalier barley the fungal development was more limited and the macroconidia production was very rare with most fungal hyphae found on the awn surface.

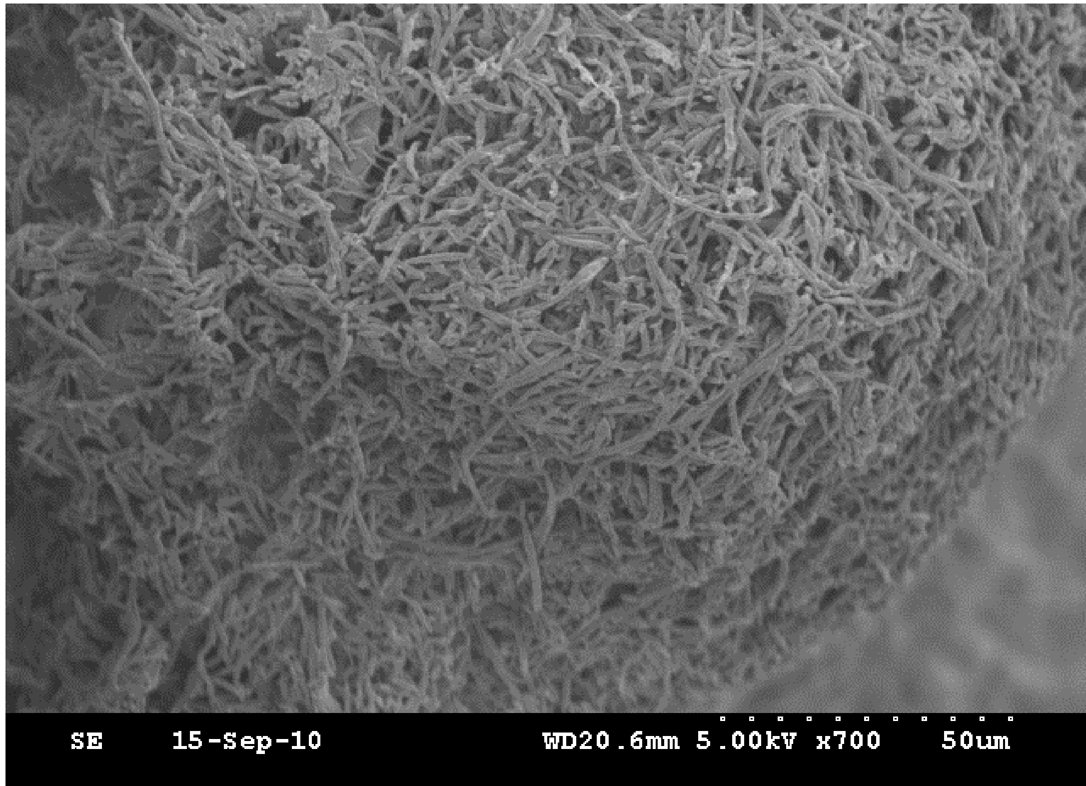


Figure 5.6: Mycelia growth covering the grain surface of Westminster barley seed.

In general, hyphal growth showed diverse patterns on different parts of the heads' outer surface with differences between varieties. For example, the hyphae and colonies in some parts of some varieties grew extensively to form continuous mycelial networks. However, the hyphal growth in other parts or on other varieties was limited with hyphae forming few or no branches during its growth.

Furthermore, SEM investigation showed that there is no entrance of the pathogen into the tissue of the heads through the stomata of the awn with hyphae observed occasionally near or over the stomata but not entering them (Figure 5.7).

Figure 5.7: Fungal hyphae crossing over awn stomata (arrows) of Tipple barley.

SEM investigation also showed fungal growth along the ventral furrow of grains (Figure 5.8).

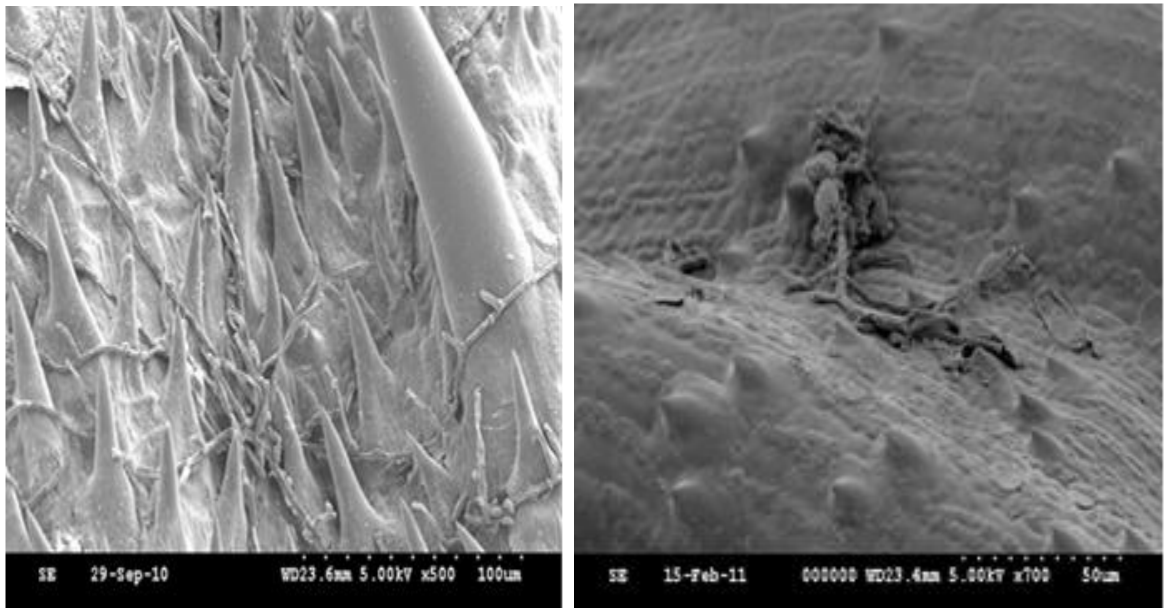


Figure 5.8: Fungal colonization along the ventral furrow of the grain on Armelle (left) and Vellavia (right).

In Tipple barley which showed high levels of FHB disease symptoms, the fungal colonization was observed on the the outer surfaces of seed especially on the seed tips and awns (Figure 5.9).

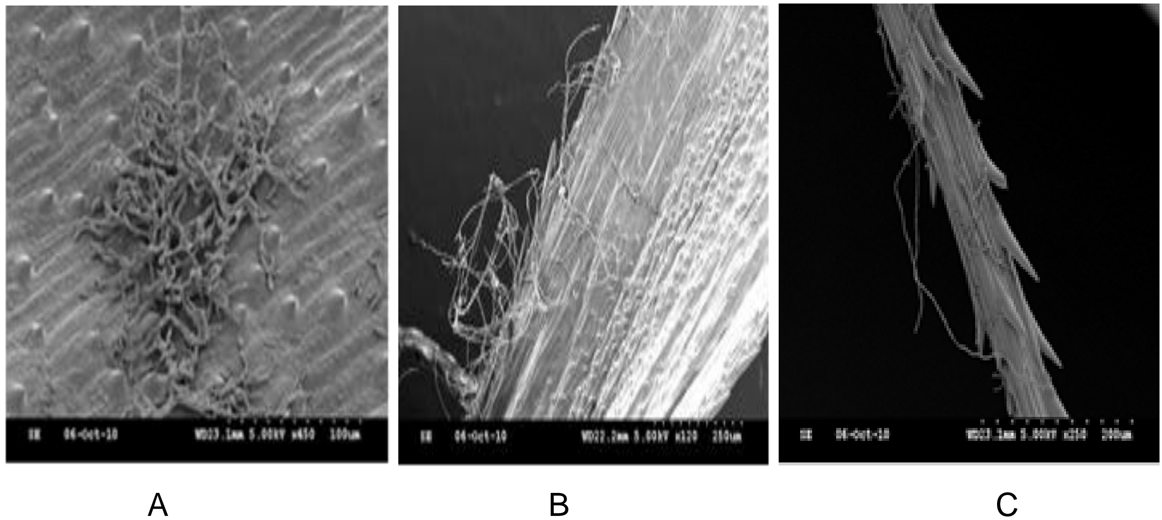
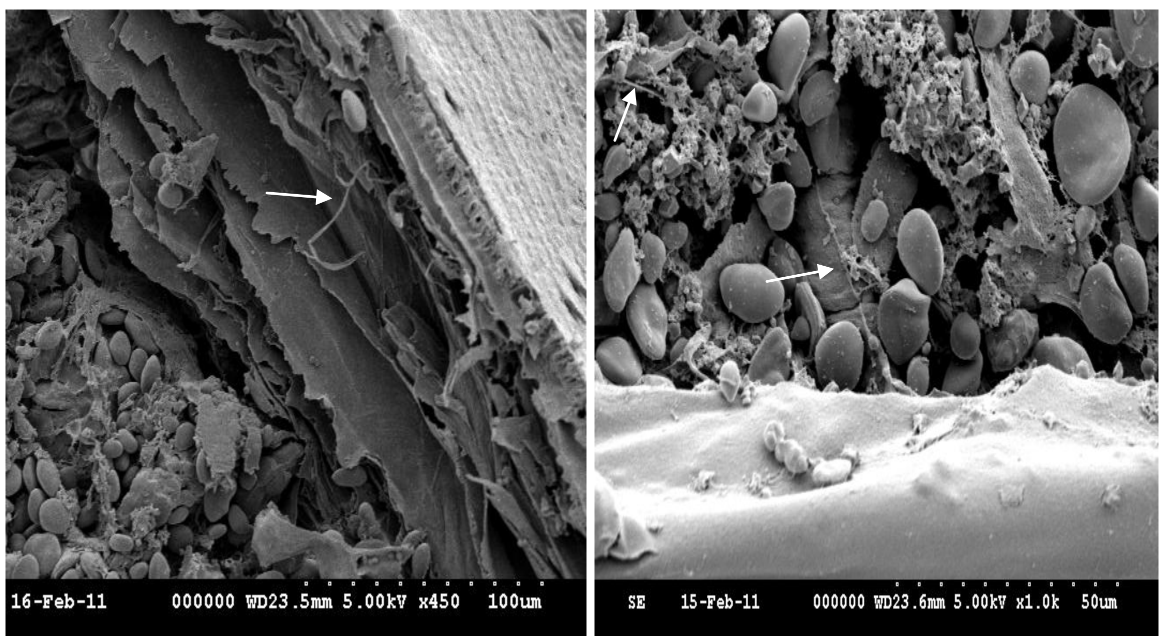


Figure 5.9: Fungal hyphae network on the outer surface of the head of Tipple barley, A- grain and B- C awn.

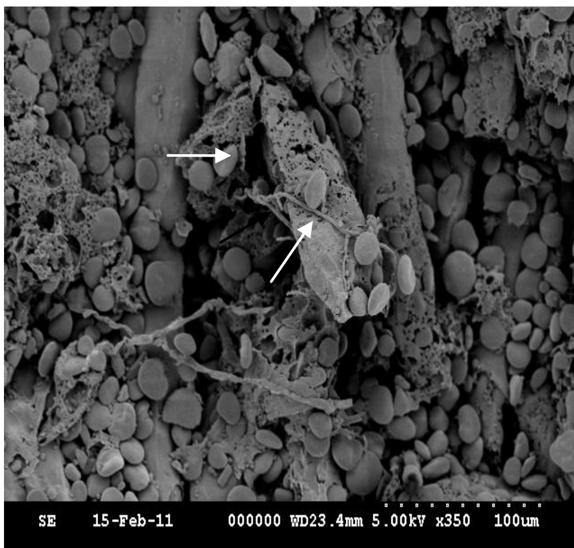
SEM investigation of longitude sections of infected barley grains revealed differences between barley cultivars in the type and extent of fungal growth in internal tissues. However, in all varieties the fungal growth within grains is lighter with shorter and thinner hyphae compared to those on the outer surface of the heads. In some cases, inoculated barley heads showed hyphal growth on the inner surfaces of the lemma and palea as shown for Armelle and Vellavia in Figure 5.10. In most cases, fungal hyphae were found just beneath the lemma or palea. No conidia were formed on the inner surfaces just conidiophores (Figure 5.10).



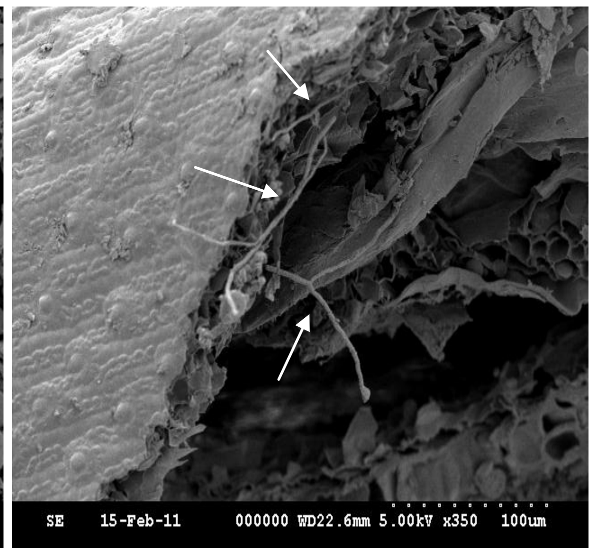
Armelle Chevalier

Plumage

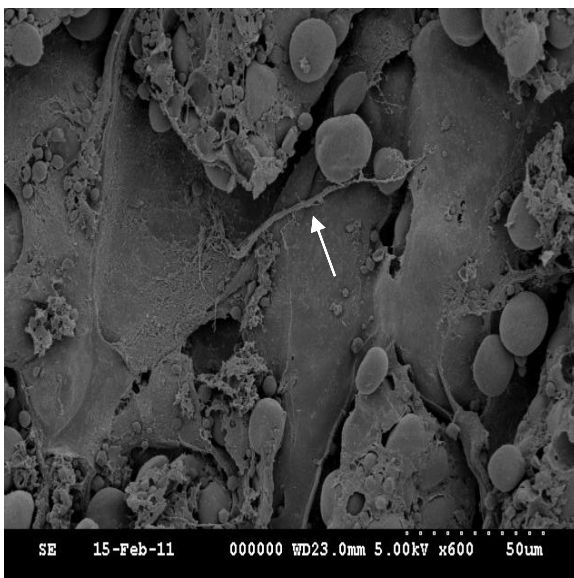
Tipple



Tipple

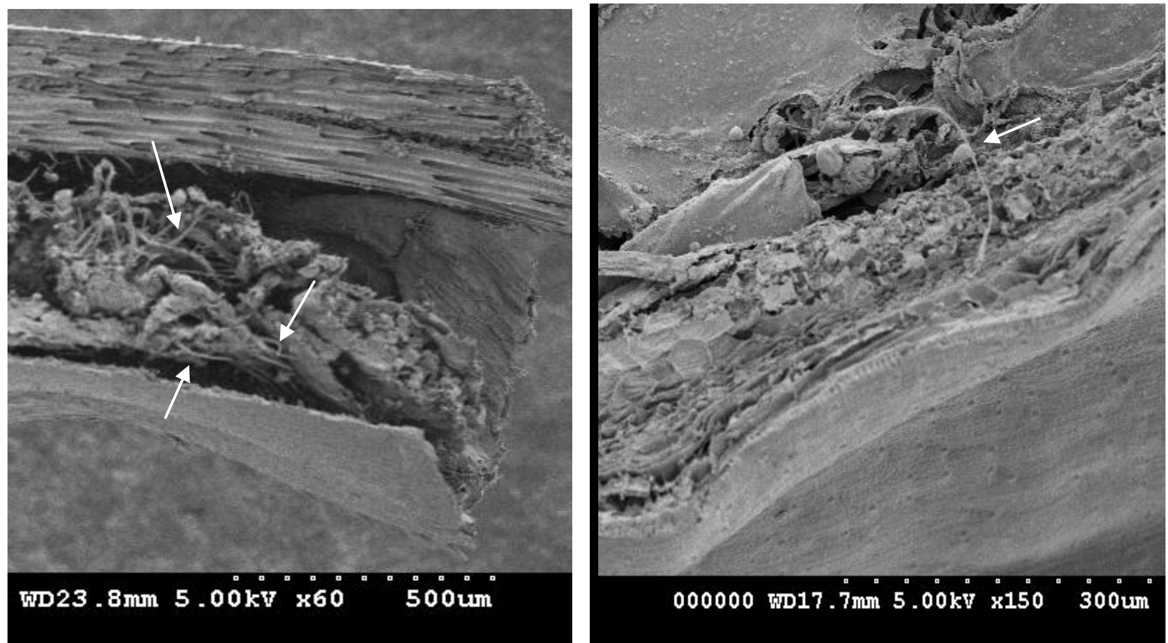


Vellavia



Vellavia

Oderbrucker

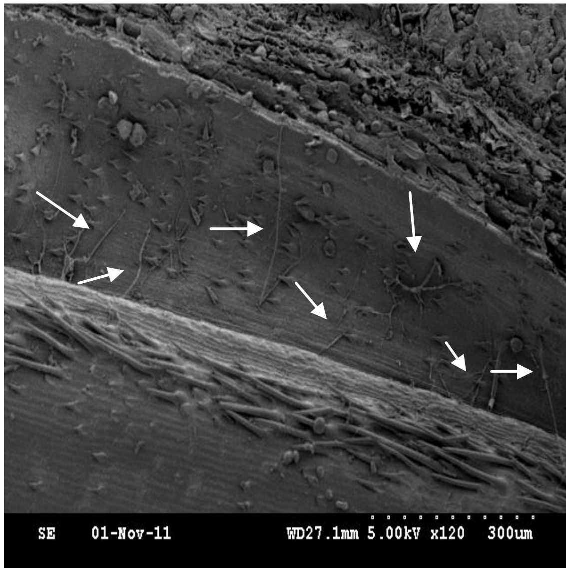


Westminster

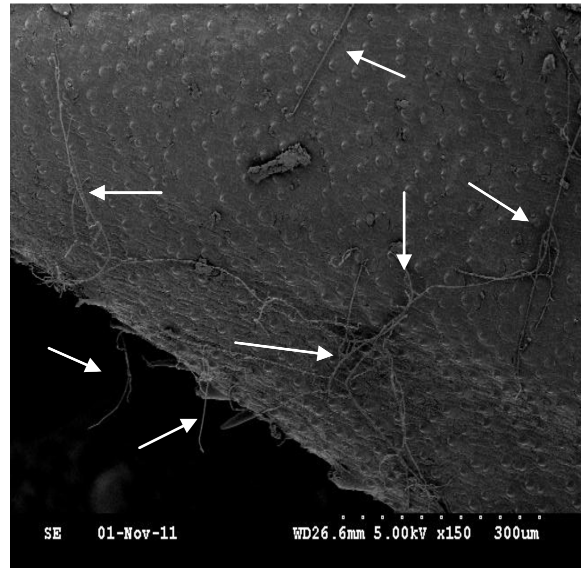
Figure 5.10: Internal colonisation of barley grains showing different characteristics of fungal hyphae growth (arrows).

5.3.2.2 Fungal hyphae growth on control seeds immersed in suspensions of *F. culmorum*.

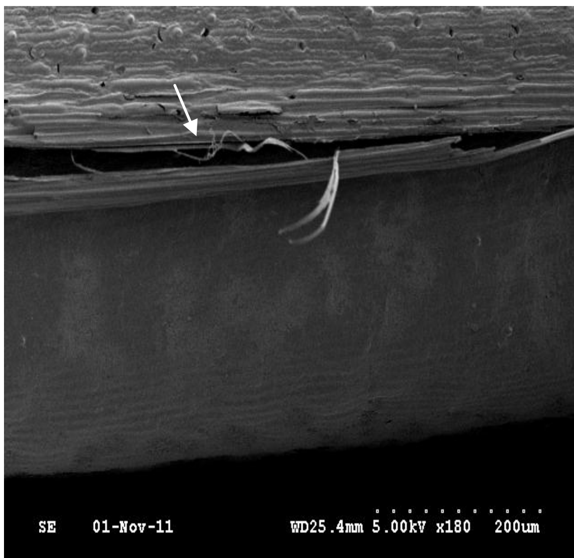
SEM investigations of the outer surface of intact barley seeds inoculated with *F. culmorum* suggested that the fungus grows differently on the barley varieties. Fungal development was observed in Armelle, Tipple and Plumage varieties particularly at the ventral furrow of grains (Figure 5.11). However, no or limited fungal growth was observed in Chevalier, Oderbrucker, Vellavia and Westminster varieties.



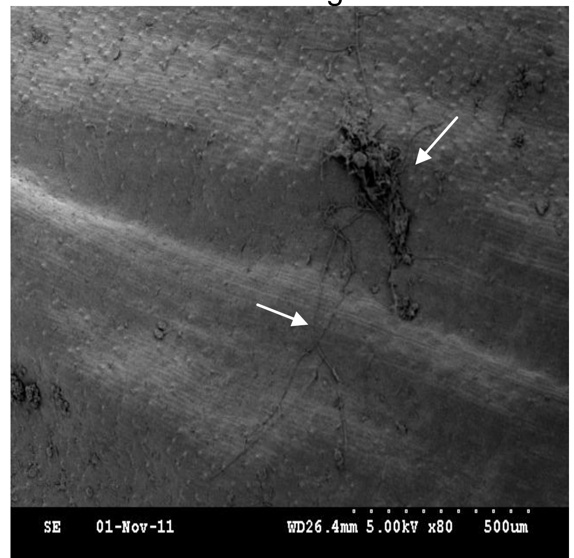
Armelle



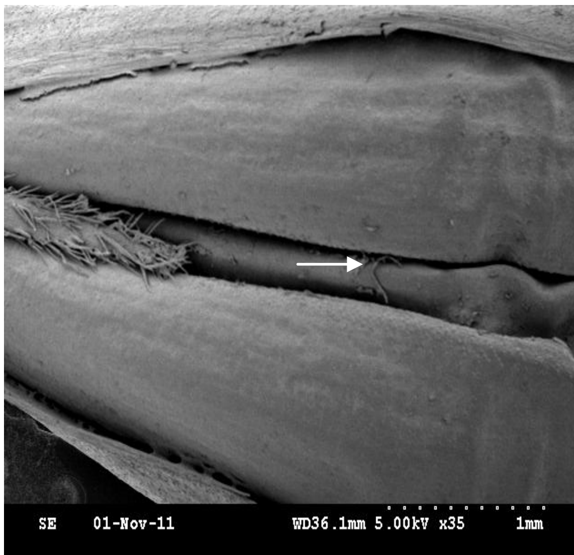
Plumage



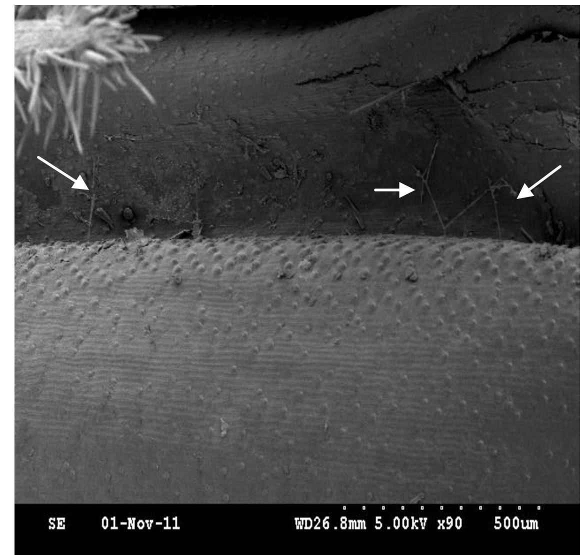
Oderbrucker



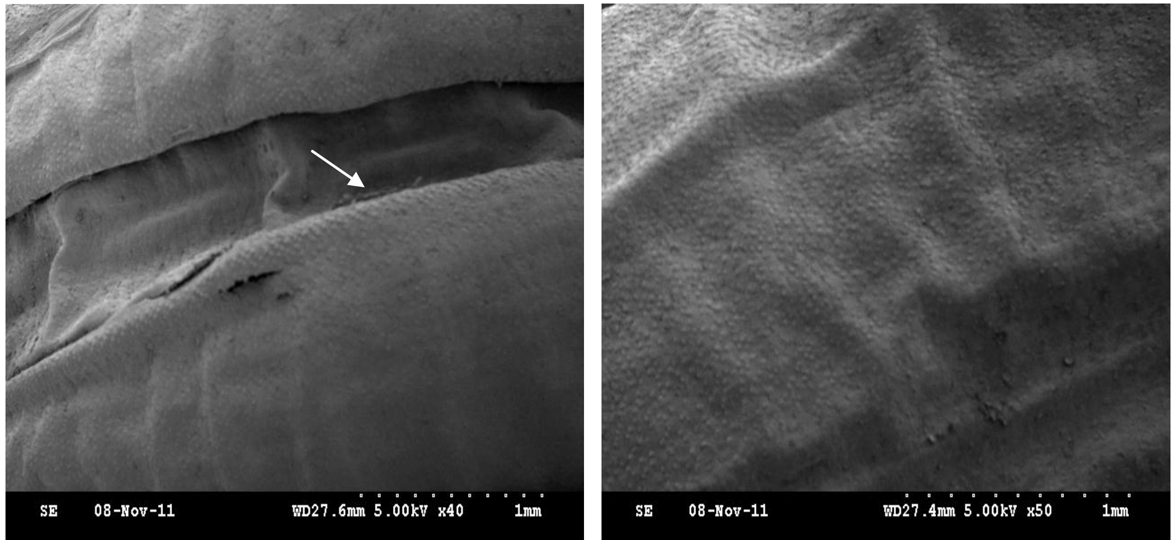
Tipple



Tipple



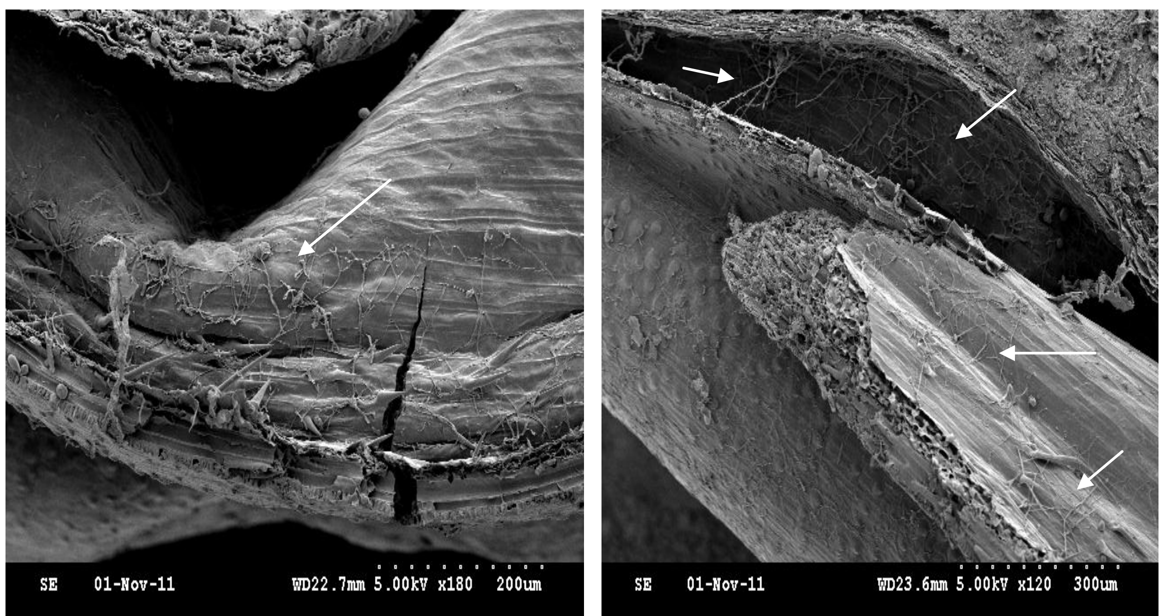
Westminster



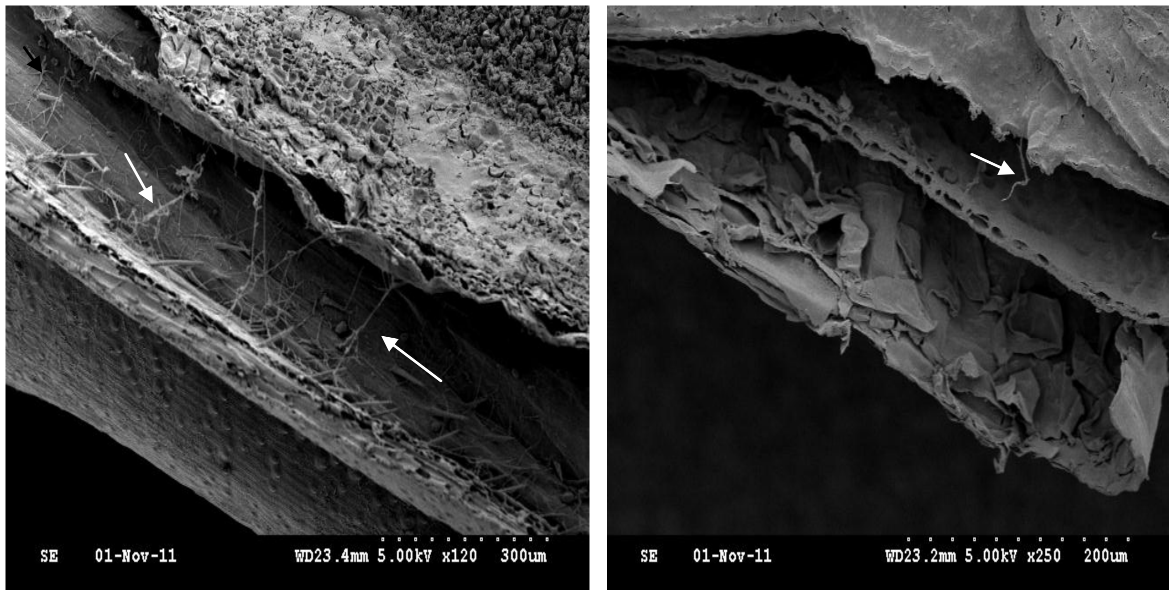
Chevalier

Figure 5.11: Fungal hyphae growth (arrows) on the outer surface of grains of different varieties 7d after inoculation

SEM investigation of longitudinal sections of grains showed differences between barley varieties in fungal growth development in internal tissues. However, in most varieties the fungal progress within grains is lighter compared to the inner surfaces of the lemma and palea. For example, heavy infection levels were observed in Westminster barley beneath the lemma or palea (Figure 5.12) compared to Chevalier, Vellavia and Oderbrucker varieties which showed no or very limited colonization.



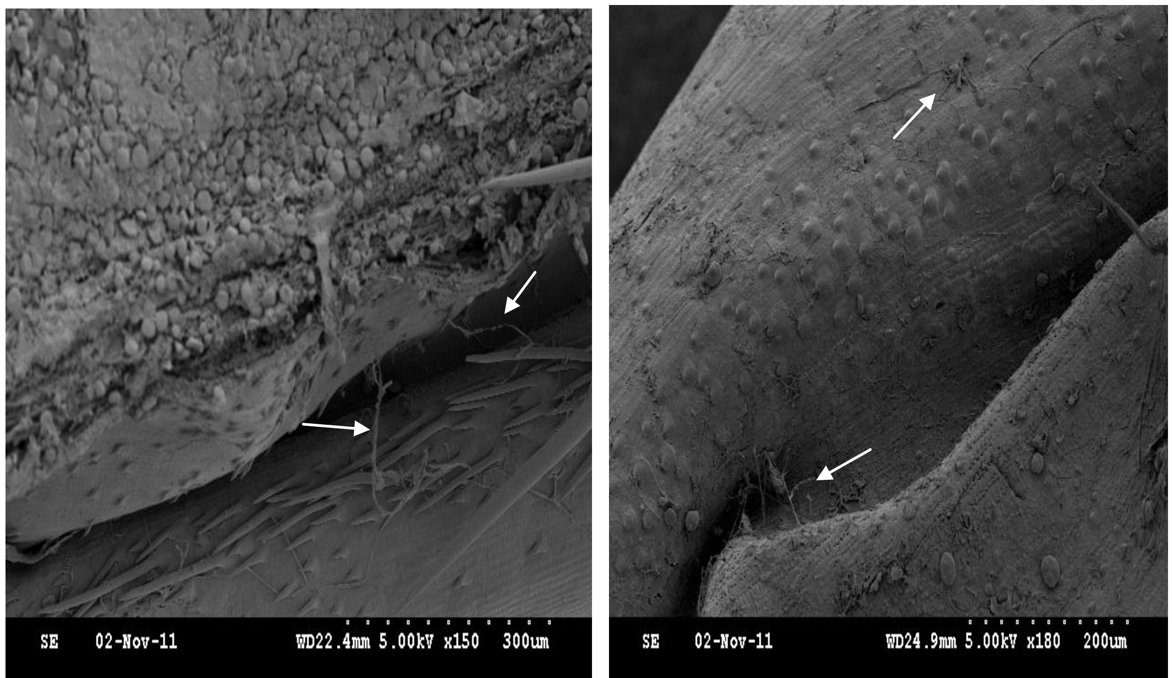
Westminster



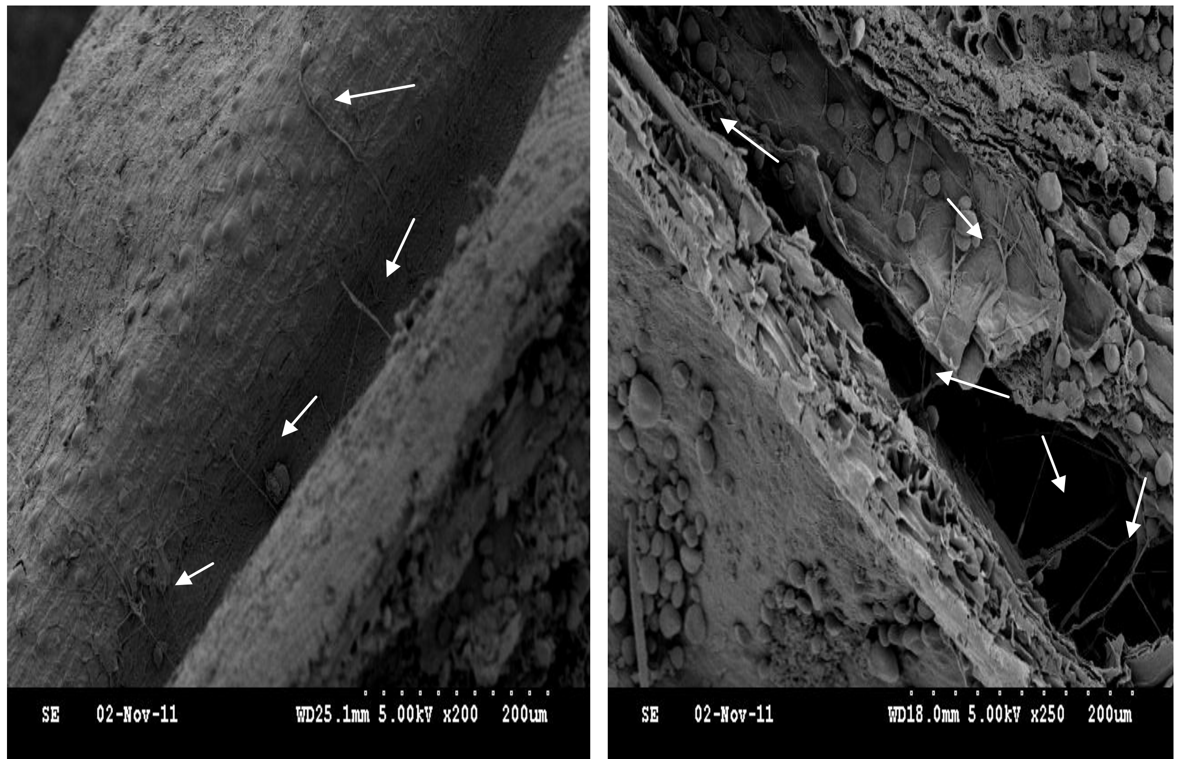
Westminster Vellavia

Figure 5.12: Internal colonisation showing different levels of fungal hyphae growth (arrows) in different barley varieties 7d after inoculation

Fungal colonization was detected in grains incubated for only 3d in Armelle, Tipple and Westminster (Figure 5.13). By 7 days after inoculation, more hyphae were seen in these varieties in addition to Plumage while no or very limited growth was seen in Chevalier, Oderbrucker and Vellavia.



Armelle Tipple



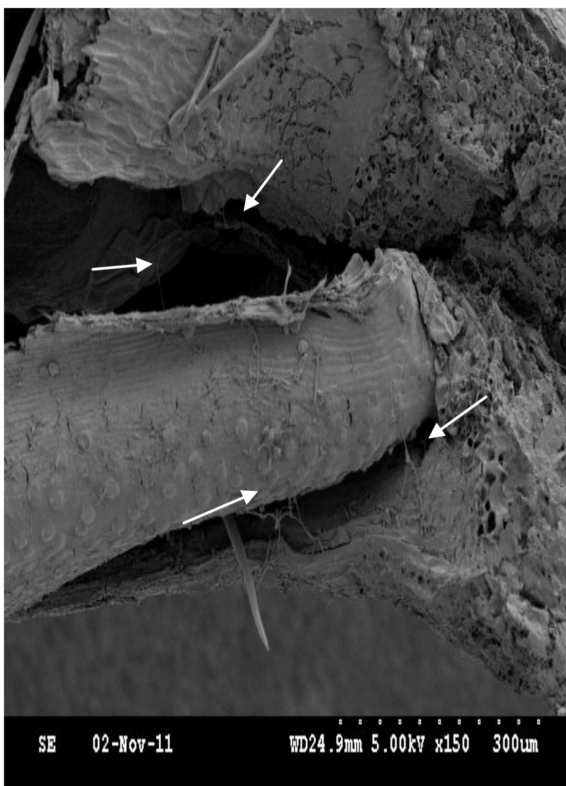
Tipple Westminster

Figure 5.13: Hyphal growth in barley seeds 3d after inoculation

The penetration by the pathogen was detected on grain tips as shown in most varieties but once again with no or limited growth in Chevalier, Oderbrucker and Vellavia. This indication confirms the previous SEM results as the conidia were found to grow near the seed tips and awns (Figure 5.14). This is illustrated in the comparison between Tipple and Chevalier.

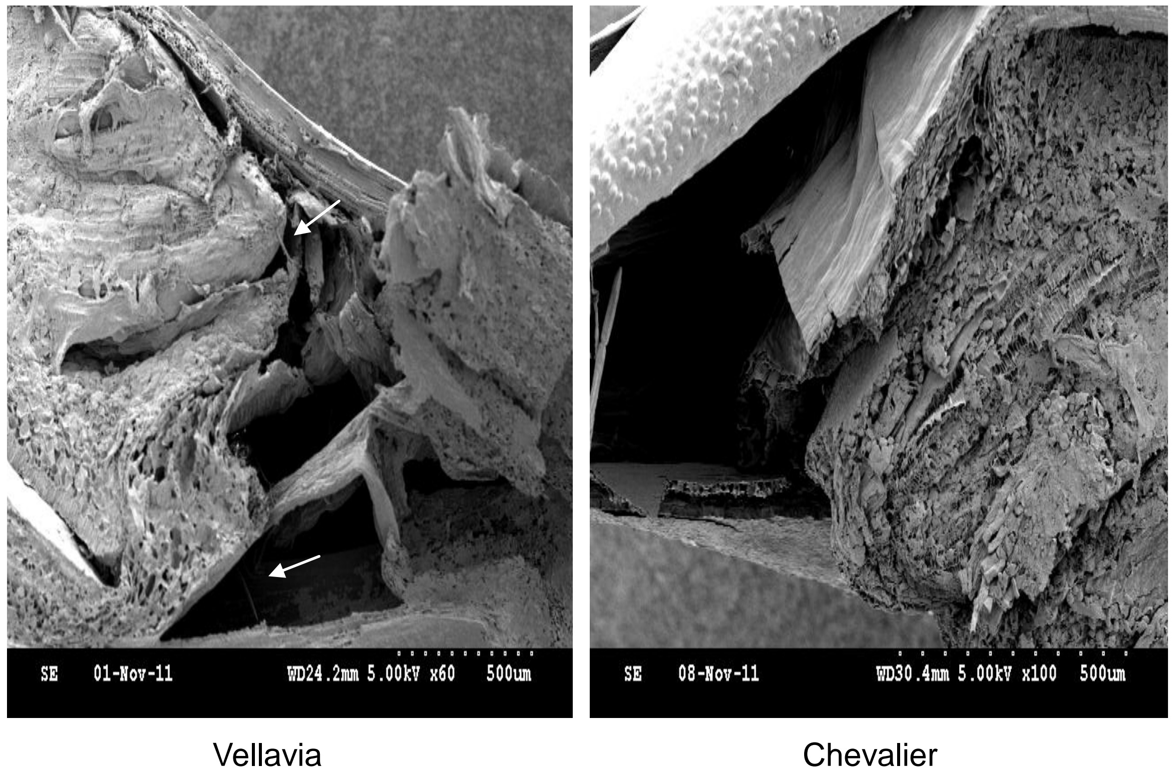
Armelle

Plumage



Tipple

Westminster



Vellavia Chevalier

Figure 5.14: Hyphal growth (arrows) in barley grain tips.

Overall, fungal colonization showed diverse patterns on different parts of the outer or internal surfaces of seeds with differences between varieties. For example, the hyphae in Armelle, Plumage and Tipple varieties grew extensively to form mycelial networks on the outer surface. However, hyphal growth on the inner surfaces was observed in Armelle, Tipple and Westminster varieties. Nevertheless, no or very limited fungal growth was observed in Chevalier, Oderbrucker and Vellavia. On the other hand, Plumage showed extensive fungal hyphae on the outer surface, whereas very limited growth was observed in internal surfaces.

5.3.3 Germination energy of barley.

Germinative energy (GE) tests were carried out in petri dishes to investigate the ability of different barley cultivars to germinate. Preliminary tests were conducted using two volumes of distilled water 4 ml and 8 ml to determine the effect of water volume. Comparison between barley samples and the percentage of germinated grains after 24, 48, 72, and 96 h are presented in Table 5.1. Chevalier, Tipple and Westminster varieties were investigated to assess the hypothesis that Chevalier

has comparable malting potential to representative modern varieties.

Table 5.1: Percentage of germinated grains of different barley varieties after 24, 48, 72 and 96 h of steeping.

Barley	4 ml H_2O					8 ml H_2O				
Variety	24 h	48 h	72 h	96 h	**Total**	24 h	48 h	72 h	96 h	**Total**
Chevalier	23	41	3	31	**98%**	77	23	-	-	**100%**
Tipple	0	4	52	42	**98%**	14	65	12	0	**91%**
Westminster	27	70	0	3	**100%**	18	49	30	0	**97%**

The results presented in Table 5.1 indicated that the germination characteristics are influenced by the quantity of water added and that germination was at different rates over the four days incubation and therefore gave a good indication of malting potential. For example, Tipple barley grains did not germinate well with 4 ml water until after 72 h and after 48 h with 8 ml water. However, GE rate for Chevalier barley was higher with 8 ml water (77%) after 24 h. In general, the highest levels of GE with 4 ml water were recorded for both Chevalier and Westminster varieties after 48 h steeping (64 and 97% respectively). However, after four days steeping, the GE was 98% for both chevalier and Tipple grains and 100% for Westminster with 4 ml water. On the other hand, the GE with 8 ml of water showed that Chevalier barley has the highest levels of GE 100% compared to Tipple and Westminster (91 and 97% respectively).

Overall, the results suggest that GE is influenced by the barley variety in addition to the amount of water added to the samples. Both GE and speed of germination are necessary for effective malting. The germination rate presented in Table 5.1 indicated that all three barley samples tested are suitable for malting. However, the relatively short germination period of Chevalier and Westminster indicated that these varieties exhibit more rapid germination and may malt better than Tipple barley.

5.4 Discussion.

The morphological study of heads conducted using light and electron microscopy illustrated the infection pattern in barley heads for different varieties. The results indicated that the heaviest infection was on the awn of barley heads even in Chevalier barley which showed lower levels of FHB symptoms on grains. Results also indicated that the grain surface can show extensive fungal growth. Conversely, less growth was seen in internal tissues both through the husk layers to the aleurone and eventually into the endosperm.

SEM investigation also presented differences in hyphal density between different barley varieties with some showing extensive mycelia in parts. The results of other studies indicated that faster colonization of barley heads occurred on the brush hairs followed by rapid fungal growth along the grain pericarp (Skadsen and Hohn, 2004). This continued to the epithelium or the internal layer of the lemma and palea and was followed by slower growth in the interior of the pericarp and testa. However, the aleurone and starchy endosperm persisted uninfected even at 16 days after infection.

SEM analysis also revealed that the penetration of hyphae was never observed through the stomata of the barley head, and in most cases the fungal hyphae crossed over or near the stomata (Figure 5.7). This is in agreement with Lewandowski *et al* (2006) who observed hyphal colonization of *F. graminearum* over stomata but failed to record any penetration.

Light microscopic investigation indicated that fungal development seems to be inhibited in the cross cell layers of the pericarp (testa) and the starchy endosperm. Lighter and shorter fungal hyphae growth was associated with limited penetration and colonization of the starchy endosperm layer. Other studies have suggested that this could be attributed to antifungal compounds in these parts of the grain or possibly because the aleurone has a barrier against fungal growth (Skadsen and Hohn, 2004). It may also result from the external surface of lemma and palea having thick-walled epidermal cells. Some difference between the densities of these layers was noted here with some varieties having a more open honeycomb

character than others (Figure 5.3). However, this characteristic did not correlate to the susceptibility of the varieties to FHB infection.

Barriers may also be caused by the interior epidermis having two to three layers of thick-walled hypodermal cells, with the interior epidermis and underlying layers being thin-walled (Lewandowski *et al*, 2006). Some differences were noted here of the separation between husk and endosperm layers (Figure 5.3).

Composition of layers may also be important. A study conducted by Skadhauge *et al* (1997) revealed that the testa of some barley phenotypes contains high levels of proanthocyanidins as inhibitors against *Fusarium* development and macroconidia formation. The aleurone and/or starchy endosperm also have some anti *Fusarium* activity which may assist in extending the period required for fungal hyphae to enter the aleurone and endosperm layers (Skadsen and Hohn, 2004). Nevertheless, SEM investigations here revealed that hyphae do enter the starchy endosperm of some barley varieties (Figure 5.10) but in low density in comparison with the external surface.

Overall, microscopic investigations showed that in some varieties such as Chevalier and Plumage barleys, the extent of growth on the fruit coat of grains and awns was less in contrast with other barley cultivars. This suggests that these varieties may inhibit or have mechanisms which limit *Fusarium* colonisation. The surface of barley grains is marked by the presence of trichomes which do differ in size and morphology (Figure 5.2). However, it is difficult to correlate this to the incidence of FHB symptoms. Oderbrucker and Armelle for instance have most prominent trichomes but have very different disease incidence. In addition the former is a six-row variety and the latter a two-row.

SEM investigation did indicate that there was limited fungal growth on the heads rachis or the racilla, suggesting that these parts may inhibit or be less conducive to fungal growth in barley and result in limiting the infection of adjacent florets. This may enhance the defence of barley against FHB disease. This is in agreement with Jansen *et al* (2005) who indicated that *Fusarium* hyphae are inhibited at the rachis and rachilla of barley heads leading to a reduction in the distribution of

infection within the head and so contribute to Type II resistance.

In general, barley grains have thick-walled epidermal cells as a protection layer against direct penetration of pathogens. However, barley varieties which have thin-walled epidermal cells are considered susceptible against fungal penetration as indicated by Lewandowski *et al* (2006). This study revealed that *F. graminearum* access the floret by two pathways; crevices between the lemma and palea and through the top floret mouth. In most cases, the macroconidia produce thin mycelial colonies in order to enter the surfaces of the lemma, palea and awn. It was observed that in barley penetration of florets occurred through the adaxial awn surface to the lemma surface in the interior of the floret as a result of greater levels of floret colonisation on apical halves in contrast to basal halves on the inner surfaces of the lemma and palea.

Overall, early cell death and rapid growth through the different layers of the fruit coat associated with infected epicarp have been observed to occur independently of the production of trichothecenes (Jansen *et al*, 2005). Hence, expression of proteins or low molecular weight compounds inhibiting the growth of the pathogen in the different tissue layers of the fruit coat are required for effective and durable resistance. Preventing infection through the fruit coat is one of the aims of breeding programs. Further aims in barley will be to focus on preventing hyphal penetration of the rachis from the outside, especially through the trichomes and by expressing inhibitors with trichome specific gene promoters (Jansen *et al*, 2005).

Overall, the potential of *Fusarium* to infect depends on the features of the grain, which differs in the organic structure and physical characters of each tissue. For example, the thin waxy layer of internal surface of lemma and palea are suggested to facilitate fungal germination and hyphal growth which leads to spikelet infection (Kang *et al*, 2004).

In conclusion, the results reported here suggested but did not confirm the cause of differences in disease resistance between barley varieties. For example, the trichomes shape of Chevalier, Tipple, Plumage, and Vellavia are approximately similar, however these varieties are different in their susceptibility to FHB disease.

Additionally, the lemma of Tipple, Armelle, Oderbrucker and Vellavia are more honeycombed while Chevalier Plumage and Westminster have a denser appearance.

Observations from SEM results of intact seeds inoculated with *F. culmorum* demonstrated that the infection process and spreading pathways are different between varieties. However, the distribution of hyphae in grains at different times of incubation demonstrated differences between varieties. The progress of infection was observed after 3d incubation in Armelle, Tipple and Westminster. However, after 7d incubation more hyphae growth was observed in these varieties in addition to Plumage, while no or very limited growth was seen in Chevalier, Oderbrucker and Vellavia. The differences between varieties could be related to different characteristics of the outer surfaces, trichome features or different thickness layers (Figures 5.2 and 5.3). Lighter and limited fungal hyphae development once again was associated with limited penetration and colonization of the starchy endosperm layer. This could be related to the presence of antifungal compounds or barriers in the aleurone layer against the pathogens (Skadsen and Hohn, 2004). Previous investigations have demonstrated that Chevalier barley had limited infection levels but interestingly the present microscopy results demonstrated limited infection progress in Vellavia which had high infection levels under infected field and glasshouse conditions. Conversely, extensive fungal hyphae were observed on the outer surface of Plumage seeds which had resistance against FHB infection under infected field and glasshouse conditions. This could be related to a different of fungal growth pattern in grains at anthesis or during milky stages of barley which is different than seeds at final growth stages which show greater hardness.

In barley, both carbohydrate and lipid exist in embryos with large quantities enough to assist as sources of nutrients during germination. Germination energy (GE) is an important factor to predict malt quality. A study conducted by Woonton *et al* (2005b) revealed that good malting quality of barley required a GE greater than 96%. In this study, the percentage of germinated grains indicated that the three barley samples tested are suitable for malting but with differences between varieties.

Results showed that Chevalier had similar germination rate in both water volumes 4 ml and 8 ml, whereas Westminster variety germinated better in 4 ml water in comparison with 8 ml water. This suggests that Westminster barley could be considered as water-sensitive. However, these results require replication to confirm.

Water sensitivity could be attributed to microorganisms present on the grains (Kelly and Briggs, 1993) and thus the microbial community may have proliferated in the 8 ml test and delayed the grain germination. These microbes can decrease the rate of grain germination through competition with the grain for oxygen. Furthermore, a higher oxygen level is required for the embryos of dormant grains to germinate in contrast with embryos of more mature grains, which become more susceptible to competition for oxygen with microbes. In general, microbes localized on the surface layers of barley grains have high oxygen uptake rates and thus competition for oxygen is a major cause of dormancy (Doran and Bricgs, 1993).

Observation of germination rates during the steeping period indicated that germination was more vigorous in Chevalier barley with 8ml of water with a higher germination rate evident after 24 h and after 48 h in Westminster and Tipple. These results are in agreement with Kitamura *et al* (1990) who noted that when barley grains are soaked in water the germination ratio increases quickly within two days and then progressively declines. A study conducted by Woonton *et al* (2005a) showed that the rapid water uptake occurred in barley up to a moisture content of 25% which influences the physical diffusion into the embryo. Thereafter, the water uptake rate became very slow but continued at a linear rate until it reached its saturation point. After that the level of oxygen intake increased with increasing moisture content especially at warm temperatures. The GE has been found to correlate with hydrolytic enzymes produced during primary stages of germination of barley during malting (McGregor *et al*, 1994).

Water uptake into the grain is a critical aspect of malting quality. Studies have shown that the differences between barley cultivars in water uptake may be related to numerous factors such as the grain size, nitrogenous content and original

moisture content of the grains. The rate of water uptake is affected by grain size as smaller grains width (less than 2.4 mm) take up moisture more rapidly and to a greater extent compared to larger grains (Molina-Cano, 1995). Thin grains have a greater ratio of husk in comparison with plump grains but less protein and starch (Li *et al*, 2008). Genetic and environmental conditions also affect water uptake (Molina-Cano, 1995). Swanston and Taylor (1990) suggested that hardness of the grain following steeping for 24 hours germination was a good predictor of malting quality.

The structure and composition of the barley grain are the factors affecting the modification of uptake. Water uptake is almost completely controlled by the endosperm hordein and β-glucan content. Differences in water uptake can be attributed to contrasts in protein quantity and quality. For example, higher water uptake and extract are associated with lower protein content and lower levels of insoluble β-glucans and B/C hordein ratio. The total β-glucan content has no influence on water uptake; however β-glucan composition and water solubility are important factors. In this case, water insoluble β-glucans may become a limiting factor by impeding water penetration into the endosperm which can act as a barrier to water diffusion throughout the endosperm, whereas the soluble fraction could act as a sponge and so enhancing water uptake (Molina-Cano, 1995).

Diastatic power (DP) is another factor assumed to largely reflect the activity of β-amylase, but is also influenced by other glycoside hydrolases such as α-amylase. The significance of DP reflects the importance of amylolytic activity and starch solublization to extract (Li *et al*, 2008). Agu *et al* (2007) reported that larger grains yield malts with greater levels of DP, and when the percentage of large grains is higher, the yields of fermentable sugars should be higher.

The amount of water is another factor affecting GE. Nowadays maltsters rely more on the 4 ml rather than the 8 ml GE assessment and use it to anticipate malting potency and speed of germination. It is the best predictor of grain germination vigour and malt quality and this is related to enzyme production capability during malting (Woonton *et al*, 2005a). There is no correlation between the 8 ml GE and malt quality however; maltsters require germination with 4 ml of water at 72 h to be

associated with uniform germination. Grains taking longer than 72 h to germinate; are considered very dormant or dead (Woonton *et al*, 2005a).

Overall, the analysis of GE reported here and SEM results of intact barley seeds inoculated with *F. culmorum* for 3d and 7d indicated that Chevalier is suitable for malting purposes and that it has limited invasion by fungal mycelium when infected by *F. culmorum* during barley growth and during storage. However, the rapid and extensive fungal colonization in Tipple and Westminster varieties indicated that these varieties are more susceptible to *Fusarium* infection during malting compared to Chevalier barley.

In conclusion, the SEM and light microscopic investigations did not reveal any specific features that could account for the differences in FHB resistance observed between varieties. However, they did confirm that growth of *Fusarium* was reduced in the resistant varieties, suggesting a physiological or biochemical cause. The results from Chapter three show that FHB resistance was associated with increased plant height and reduced tillering, suggesting a correlation with growth characteristics. The study did not reveal significant differences in the grain structure between varieties, and showed that Chevalier has excellent germination energy. This combined with the high level of *Fusarium* resistance should make Chevalier a useful variety for breeding if these positive features can be combined with short straw characteristics.

Chapter Six

General discussion, conclusions and future work

6.1 General discussion.

The purpose of this study was to characterise selected historic barley varieties in relation to their growth features, their response to nitrogen and their resistance to two common barley diseases with different aetiologies, mildew and Fusarium Head Blight. Nine two-row and four six-row historic varieties were chosen and assessed along with two elite modern varieties Tipple and Westminster, currently used for malt production in the UK.

Many historic varieties have been discarded in favour of varieties with better yield or improved agronomic performance such as ease of mechanical harvesting and resistance to lodging. However, the possibility that early varieties carry potential genetic resources for future development suggests that these varieties should be re-evaluated.

The hypotheses of this study is that some of the historic varieties chosen will demonstrate comparable or better productivity and disease resistance to elite modern varieties. A focus on malting varieties extends this hypothesis to proposing that some of these varieties will have comparable or better malting potential based on historical records.

Assessments of productivity in initial screening field trials in 2009 indicated that yield as measured as grams of grain per plant and 1,000 grains weight varied four fold and two fold respectively across the fifteen varieties assessed (Table 3.14). However, although modern varieties did produce higher yields than most historic varieties, this was not uniquely so with many historic varieties outperforming Tipple. This could be related to these varieties producing greater tiller numbers as the relationship between tiller number and grain yield and 1000 seeds weight is significant ($P \leq .001$ and .003 respectively). However, a poor relationship was found between grain yield and plant height.

Powdery mildew (natural infection) appeared on plants with high-levels on historic varieties at seedling stages however, modern varieties and Plumage historic variety showed resistance against mildew which could be related to modern varieties (Tipple and Westminster) having the mlo gene (HGCA, 2010).

Nevertheless, there was no relationship between mildew severity and varieties in the prediction of the grain yield. Mildew disease had no significant effect on grain yield. This could be related to the decrease in mildew incidence during barley growth and its disappearance at the adult stages even on susceptible varieties.

Analysis results of N content in seeds from the field experiment in 2009 revealed that modern varieties and most historic barley varieties had acceptable N content for malting purposes. Modern varieties and Chevalier historic variety had significantly lower levels of nitrogen ($\leq$ 1.32%) compared to six row varieties particularly Asplund and Dore which had significantly higher levels of N ($\geq$1.85%) (Table 3.14).

Further analysis indicated that the difference in yield between varieties depended on the level of nitrogen provided (Table 4.5). Nitrogen was found to affect a number of plant growth features including tiller number and plant height which could contribute to differences in grain yield (Table 4.9).

Disease resistance is a major concern to agriculture and to plant breeders and provides strong motivation to programmes developing new resistant varieties. The two diseases assessed in the varieties investigated here have very different characteristics, mildew being a leaf and stem disease and FHB predominantly a grain disease.

In two growing seasons, clear differences were found for the level of *F. culmorum* infection between historic and modern spring barley cultivars. In 2009, the highest levels of infected heads occurred naturally in six-row barley cultivars with the highest level 30.58% in Dore (Table 3.4). This is in agreement with Steffenson and Scholz (2001) who indicated that the resistance against FHB in six-row barley (both spring and winter types) is very rare. The relationship between powdery mildew disease appearance on most barley cultivars at seedling stage and varieties in predicting the percentage of infected heads is not significant (p = .946). Mildew had no significant effect on FHB incidence (p= .468). The reason for this could be related to decreases in mildew levels progressively during time and its disappearance at the adult stages of barley.

In 2010 field experimental results at JIC in Norwich, indicated that there was no noticeable FHB disease symptoms in control plots (not artificially infected) in contrast with high natural infection levels in Nafferton farm in 2009. This may perhaps be related to the environmental conditions during the ripening period of barley especially when it was very humid. Temperature is also a critical factor affecting FHB disease. For example, at higher temperatures the disease progress is accelerated and associated with earlier necrotic heads compared to development at lower temperatures. The effect of temperature may be on fungal development or on the host which may become more susceptible at the higher temperature. Alternatively, the effect may occur simultaneously on both the fungus and the host (Brennan *et al*, 2005). Under FHB infected conditions, the highest level of infected heads was 86.4% in two-row modern variety Tipple, with the exception of Chevalier barley the level of symptoms was 16% of the infected heads (Table 3.6).

The repeat FHB experiment with seven barley varieties under glasshouse conditions, 2010 in Sunderland confirmed the previous results. The highest levels of FHB incidence ranged between 52.84-58.61% and between 92.09-94.28% in Tipple and Vellavia for the percentage of infected grains and heads respectively. However, Chevalier barley again showed resistance against FHB disease with low infection levels (5.73 and 30.92%) for the percentage of infected grains and heads respectively. The relationship between powdery mildew disease appearance on most historic barley varieties in the prediction of the percentage of FHB infection once again is not significant (p = .632). Mildew had no significant effect on FHB level (P = .700). However, the results from nitrogen experiments, suggested that the FHB infection levels increased with increasing applied nitrogen from 0.5 to 10 mM (Table 4.8).

Mycotoxin analysis revealed low relationship between DON levels and visual symptoms. For example, levels of FHB in Tipple are high while DON levels measured by the ROSA method are lower than expected. Nevertheless, in Chevalier barley which appeared symptomless, mycotoxin analysis revealed higher levels of DON than expected. This could be probably related to other *Fusarium* species that were also causing FHB infection. The results are supported by previous investigations of Liu *et al* (1997) and could be attributed to the

environmental conditions that can have an impact on the DON production separate from fungal development. For this reason, DON is not usually an indicator of fungal biomass. However, regression analyses revealed a positive relationship between DON levels and *Fusarium* DNA (Figure 3.10).

In this work, results from both growing seasons and under different locations and conditions indicated that the varieties Tipple, Vellavia, Westminster and Asplund were most susceptible to FHB as indicated by a greater disease incidence or by higher mycotoxin levels recorded. Whereas, Chevalier, Plumage and Armelle varieties demonstrated more limited symptoms of fungal growth or lower mycotoxins levels indicating that these varieties have a greater degree of resistance against this disease. The resistance of varieties against the disease may be attributed to diverse mechanisms present in these cultivars and it is not known whether this is due to different in head morphology, antifungal components or to the presence of resistance genes or a combination of these.

Traits such as plant height, flowering timing and duration, awn absence or presence, grain density, extent of flower opening and barley row type influence resistance to *Fusarium* damage and to DON levels (Bai and Shaner, 1994; Yoshida *et al,* 2005). Bai and Shiner (2004) recognized various QTL on chromosome 2H for reducing FHB damage, DON content and grain discoloration which could be exploited to enhance resistance against FHB in barley. Furthermore, waxy surfaces on head tissue could reduce water availability to *Fusarium* conidia and thus contribute to Type I resistance (Yoshida *et al*, 2005). The phenylpropanoid and phenolics contained in the cuticular wax and or cutin may offer some protection against *Fusarium* (Jetter *et al*, 2006).

Differences between barley varieties in response to FHB disease could be related to plant height, taller varieties are more resistant against FHB disease in contrast to shorter varieties as shown by the regression in Figure 3.14. This feature is particularly evident in resistant varieties Plumage and Chevalier compared to short modern barley varieties Tipple and Westminster. Exceptions to this trend are evident for example Armelle which is considered as a short variety but demonstrated resistance against FHB and Vellavia showing medium height, but a

high level of infection. The possibility of these varieties carrying different genetic resistance mechanisms should be considered for future investigations.

In other analyses, most two-row barley varieties which produce higher tiller numbers are more susceptible to FHB disease as is evident in modern varieties Westminster and Tipple and the historic variety Vellavia (Figure 4.15). In contrast, Chevalier barley which has lower tiller numbers is associated with lower levels of disease infection. This could be attributed to a higher humidity associated with greater tiller numbers providing conditions more favourable to fungal development. Moreover, most of six-row barley varieties used in field experiments in 2009 are susceptible as evident in varieties Oderbrucker, Asplund, Dore, and Bigo (Table 3.4). These showed higher levels of FHB disease compared with other two-row barley varieties which could be related to the greater aeration in two-row barley heads compared to the six-row barley varieties.

Cleistogamous flowering type could be an important characteristic for disease resistance and most two-row barley varieties have been considered as cleistogamous (Yoshida *et al*, 2001). In general, resistance against FHB can include different responses and plant physiology. For example, it has been found that barley varieties produced heads at different times (Table 3.8). The different flowering dates or flowering periods could be considered reasons for different levels of infection between different barley varieties. However, the duration of anthesis was not assessed and experiments to identify the flowering times provide suggestions for further research. The suggestion of plant resistance against FHB disease could be related to a short flowering period and this could be considered as escape rather than true resistance and linked to mechanisms to prevent conidia reaching the heads. Identifying genes controlling flowering times would be useful for future developments of resistant varieties.

In summary, comparisons between barley varieties revealed a greater FHB disease incidence in the modern varieties and in Vellavia historic barley variety (Table 3.7). Two-row barley varieties appear to have an inherent Type II resistance (Langevin *et al*, 2004; Foroud and Eudes, 2009) which could limit spread between grains and it would be interesting to test this for these varieties using point inoculation of seeds as well as to assess whether any varieties

possess Type IV or Type V resistance by determining ability to detoxify trichothecenes (Boutigny *et al*, 2008).

Some barley varieties studied here showed different patterns of infection with artificial inoculation under glasshouse conditions compared to field conditions. For example, Armelle barley presented good resistance against FHB disease under field conditions at JIC. However, under glasshouse conditions this variety was susceptible and as a result recorded greater disease symptoms. These contrasting results may be attributed to the different environmental conditions such as temperature between the field and the glasshouse. A greater infectivity at higher temperatures may be a relevant consideration when selecting varieties for use in climate change conditions.

Observation of infected barley heads was conducted to assess the extent of fungal presence on and in grains. Overall SEM of barley heads indicated differences in fungal colonization between different barley varieties and between the different parts of the same head. For example, a greater hyphal density was observed in most barley varieties while in Chevalier limited fungal colonies were observed with reduced spreading of fungal hyphae (Figures 5.5 and 5.10). This suggested that Chevalier has resistance to the growth of the fungus. However, the different resistance between barley varieties could be related to the different mechanisms controlling the response against this disease such as trichome length and morphology, husk thickness and grain hardness.

Observation of longitudinal sections of barley grains showed only a low-density of hyphae in internal tissues which may be related to the other possible resistance mechanisms. These mechanisms may result from inducers produced during fungal development in the host plant providing resistance activation. However, the colonization of fungus on the glume, lemma, palea and brush hairs suggests that there may be few inhibitors on these parts of barley heads. A study conducted by Bushnell *et al* (2001) proposed that the epidermis of the outer surfaces of the florets and the glumes of barley consist of very thick-walled cells. Direct penetration through these reinforced cells by *Fusarium spp* is difficult. However, each of the glume, palea and lemma layers that enfold the floret have numerous rows of stomates which can be entered by *Fusarium* hyphae. However, fungal

invasion via stomatal entry is unclear. This study also indicated that the mouth at the apex of the floret is another possible pathway of fungal entry and the fungal hyphae can also colonize interior surfaces of the palea and lemma. Within tissues it has been found that *F. graminearum* can grow between cells instead of entering them and establishing a biotrophic relationship with host tissues (Bushnell *et al,* 2001).

Skadsen and Hohn (2004) proposed that a low-density of fungal hyphae could be attributed to antifungal inhibitors present in the endosperm and embryo tissues which might hinder the fungal growth directly and enhance barley resistance against FHB disease. Studies conducted by Nuutila *et al* (1999) also indicated that barley has antimicrobial materials such as thionins which have a role against fungi.

Internal tissues have a limited defensive role at early phases of infection, but could impede fungal development and its access to nutrients. It is not known whether inhibitors have direct antifungal action or inhibition of proteinase activities. It would be more beneficial if the inhibitor production occurs in the husks of grains although this may affect the germination of the seed.

Overall, preventing infection through the fruit coat is one of the aims of breeding programs. Further aims for barley, will be to focus on preventing hyphal penetration of the rachis from the outside, especially through the trichomes by expressing inhibitors with trichome specific gene promoters (Jansen *et al*, 2005).

A study conducted by Pekkarinen (2003) revealed that fungal toxins have a role in the FHB infection progression, but also that pathogens can produce a number of hydrolytic enzymes and hormone-like compounds which also impact on the invasion rate. For example, proteinase enzymes have a role in degrading plant proteins to provide nutrition to support pathogen development. Observations of digestion of cell walls and protein matrices and the lack of digestion of starch grains in some varieties (Figure 5.10) suggest that proteases and glucanases, but not amylases may be active. Further analysis of infected grains for these enzymes could illustrate possible resistance mechanisms.

Resistance to FHB infection of mature grains is a further consideration and is relevant to the storage of barley in preparation for malting. The results reported here on the distribution of surface fungi are relevant to contributions these may make to growth during storage and malting. For this reason, further SEM investigations of the outer and internal surfaces of intact barley seeds inoculated with *F. culmorum* for 3d and 7d incubation were conducted on the same varieties. The results suggested that *F. culmorum* had different pattern on mature grains compared to non-mature grains. Fungal colonies were observed on the outer surface of Armelle and Tipple particularly at the ventral furrow of grains. Plumage barley was considered as a resistant variety as low infection levels and limited fungal hyphae recorded in this variety in initial observations. However, this variety showed a high density of fungal hyphae on the outer tissues when uninfected grains were inoculated with *F. culmorum* (Figure 5.11). On the other hand, no or limited fungal growth was observed in Chevalier, Oderbrucker, Vellavia and Westminster varieties although these varieties with exception of Chevalier showed moderate to high levels of FHB incidence.

Further SEM investigations of longitude sections of grains also showed differences between varieties. In most varieties, the fungal progress within grains was lighter than the inner surfaces of the lemma and palea. Heavy infection levels were observed in Westminster barley beneath the lemma or palea while again Vellavia and Oderbrucker varieties showed very limited colonization (Figure 5.12). However, once again no fungal colonization was noticed in Chevalier barley. This could be related to different mechanisms, inhibitors, antifungal or genes controlling the response against FHB disease which may be activate during barley growth stages.

Investigation of the differences in malting quality between the historic variety Chevalier and modern varieties Tipple and Westminster revealed that all these varieties have good malting properties. However, these varieties showed differences in their germination energy (Tables 5.1), for example Chevalier barley had the highest level and the faster germination rate after just 24 h compared to Tipple and Westminster barleys. On the other hand, Westminster barley is regarded as water-sensitive as a result of better germination in 4 ml water compared to 8 ml water. Nevertheless, Chevalier variety showed similar patterns in

both 4 ml and 8 ml of water. Overall, Chevalier barley showed the highest germination levels overall and a faster germinating rate indicating that this variety is at least as favourable for malting purposes as Tipple and Westminster and so confirms the hypothesis proposed.

Furthermore, the total nitrogen values of Chevalier barley seeds harvested under normal conditions in Nafferton farm in Newcastle in 2009 had low nitrogen values similar to Tipple and Westminster varieties (Table 3.14) and as required for malting. In general, Chevalier, Tipple and Westminster varieties had a high germination rate over the same period of germination which may suggest that low nitrogen enhances germination. This is supported by studies by Agu and Palmer (2001) who indicated that the degree of physical alteration of the endosperm occurs earlier in barley with low nitrogen levels. While the germination rate is high in these varieties, SEM results of intact barley seeds inoculated with *F. culmorum* for 3d and 7d indicated that Chevalier is more suitable for malting purposes as no fungal colonization observed in this variety compared to Tipple and Westminster samples which showed rapid and extensive fungal colonization.

Although field grown Chevalier produced grains with low levels of nitrogen, results from investigations of the effect of nitrogen on barley growth indicated that this variety accumulates more nitrogen in its grain compared to Tipple (Table 4.6). These results indicated that this variety responded well towards nitrogen even at low levels. The present results could be attributed to Chevalier barley being able to produce good quality grain on unproductive soils and unsuitable to grow on clayey soils because of the weakness of the straw (Milburn, 1843). The present results suggest the importance of controlling the nitrogen level to obtain high yield combined with good malting quality.

A summary of results of Chevalier barley compared to Tipple and Westminster in response to nitrogen use effects on plant height, tillering, yield, nitrogen content in grain and percentage of infected grains with FHB are presented in Table 6.1.

Table 6.1: Effect of nitrogen levels on historic and modern barley varieties.

Variety	Height	Tiller number	Yield/plant (g)	Nitrogen in grain	% of infected grains with FHB
			0.5 mM		
Chevalier	61.75	2.5	1.58	1.77	1.19
Tipple	43.5	2.75	2.22	1.26	16.31
Westminster	58.5	3.5	3.11		14.25
			1 mM		
Chevalier	85.75	2.75	2.45	1.88	1.8
Tipple	58	3.5	3.37	1.34	33.92
Westminster	57.5	5.25	4.28		33.04
			2.5 mM		
Chevalier	95.25	5.25	5.68	1.96	17.87
Tipple	62.5	7.25	6.03	1.81	27.45
Westminster	68	8.5	5.55		27.93
			5 mM		
Chevalier	87.25	7.5	5	2.61	28.16
Tipple	63.25	11.75	5.60	2.20	62.83
Westminster	66.5	14	6.85		39.6
			10 mM		
Chevalier	77.25	10.25	2.60	2.79	64.87
Tipple	58.25	20.25	5.90	2.58	83.24
Westminster	59.25	19.75	5.88		61.11

All these results indicate that the historic variety Chevalier which first appeared more than one hundred years ago has desirable characteristics for growers of malting barley. Besides a suitable yield and grain nitrogen level a major feature is resistance against FHB disease causing considerable economic losses to growers in addition to mycotoxin production. Furthermore, Chevalier has high levels of germination energy which is a desirable feature in produce high quality malted barley.

Unfortunately, this variety is very susceptible to powdery mildew disease particularly under glasshouse conditions, most likely because this variety does not contain the mlo allele a feature only discovered 50 years after Chevalier became obsolete.

The high-level of mildew causes a reduction in the level of photosynthesis in plants (Swarbrick *et al,* 2006) in addition to the disease effects in reducing tiller and grain

number and grain size (Scott and Griffiths, 1980). The present results do not agree with Scott and Griffiths (1980) as ANCOVA results indicated that mildew disease had no effect on the number of tillers, dry weight and grain yield. However, the results from nitrogen experiments indicated that mildew disease incidence increased with increasing applied N levels which could be related to nitrogen effects on the production of soft tissue with little resistance to penetration by fungal hyphae (Krauss, 1999). It could also be related to increased plant growth and humidity which is more favourable to fungal growth (Last, 1962b) or increasing colony density with increasing N application by enhancing the ability of colonies to produce more spores (Jensen and Munk,1997; Sander and Heitefuss,1998) .

Although the current study did not confirm the reason for the high resistance levels against FHB infection in Chevalier barley, it has provided information to support breeding programs. These could usefully investigate resistance genes in this variety particularly as Chevalier has rapid and high levels of germination energy and was the favoured malting variety in the nineteenth century. Therefore, it may be feasible to develop varieties with good resistance against FHB disease associated with higher yield production and good malting quality. Chevalier is also a tall variety and not acceptable for modern agriculture due to its lodging in addition to produce fewer tillers.

The present study sought to answer the research questions raised in Chapter one. The series of experiments was conducted to answer these questions by using different varieties of barley. The data presented here revealed information and suggestions relevant to the research questions. For example, low levels of FHB symptoms and SEM investigation results indicated that some historic barley varieties have more resistance to FHB disease in contrast to contemporary varieties. SEM results indicated that the awns could be considered as an infection pathway in barley. However, SEM results did not confirm the factors affecting the resistance features of barley against FHB disease, but gave suggestions that resistance mechanisms could be related to a physiological or biochemical cause. On the other hand, the results presented in Chapter three show relationships between FHB resistance and growth characteristics of barley such as plant height and tiller number.

The importance of the research question was seen to extend beyond the relative efficiencies of resistance varieties to incorporate into future breeding trials programs by crossing to develop new FHB resistant varieties combined with greater yield and good quality of malt.

For future development, the mlo allele and short straw length could be bred into modern high-yielding barley. Crosses between Armelle and Chevalier with Tipple have been initiated at JIC as a first step to reduce the susceptibility of Tipple barley against FHB disease, and to investigate the genetics of the trait. Identification of resistance mechanisms to FHB may also be of value for other crops such as maize which may contribute to long-term infection levels as a low-level reservoir in crop residues during crop rotation (Foroud and Eudes, 2009).

The results reported here provide preliminary suggestions for further research to investigate the characteristics of Chevalier both for disease resistance and malting suitability. These will require a larger quantity of grains and to obtain these stocks must be grown. More research is also required to determine if there is potential to make crosses between Chevalier barley with modern varieties to maximise yield and to decrease levels of FHB disease and mycotoxins.

A general conclusion is that the original hypothesis appears to be valid in so much as that an understanding of the physiology and disease susceptibility of different cultivars is important for selecting cultivars for useful characteristics in future breeding programmes.

However, it is also clear from this study that in hindsight the hypothesis underestimated the number of key variables that determine, in particular disease resistance. Literature analysis and input from collaborators indicate that flowering onset and duration require more detailed analysis. Assessment of the data reported here suggests that flowering onset (and potentially duration) may affect susceptibility to FHB in both the field and under glasshouse conditions and warrants more serious investigation in the future. However, this work does add useful data to our understanding of the complexity of plant physiology and cereal yields under varying environmental conditions and provides some useful directions for further study into disease susceptibility.

6.2 Future work.

- Further experiments to assess the duration of anthesis and to identify the flowering times which may affect susceptibility to FHB.

- Identifying genes controlling flowering times would be useful for future developments of resistant varieties.

- It would be interesting to test for an inherent Type II resistance in varieties studied here by using point inoculation of seeds as well as to assess whether any varieties possess Type IV or Type V resistance by determining ability to detoxify trichothecenes.

- Further assessments of physiological or biochemical resistance mechanisms in Chevalier barley.

- Further analysis of infected grains for proteinases, glucanases and amylases enzymes which have a role in degrading plant proteins could clarify possible resistance mechanisms.

- Identification of resistance genes in Chevalier barley which has good resistance against FHB disease and good malting quality so as to develop more manageable varieties with these characteristics.

- More research is required to determine if there is potential to make crosses between Chevalier barley with modern varieties.

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Appendix

Appendix.

Table A1: Percentage of leaf area covered with mildew and percentage of infected plants in historic and modern barley varieties in the field, 2009 (F 2009).

23-6-2009 (second score)				10-7-2009 (final score)		
		% of infected area -upper leaves	% of infected plants		% of infected area-upper leaves	% of infected plants
Asplund	(6)	0	0	Armelle	0	0
Bigo	(6)	0	0	Dore	0	0
Chevalier	(2)	0	0	Hannchen	0	0
Loibichl	(2)	0	0	Plumage	0	0
Nottingham	(2)	0	0	**Tipple**	**0**	0
Oderbrucker	(6)	0	0	Union	0	0
Plumage	(2)	0	0	Vellavia	0	0
Tipple	**(2)**	0	0	**Westminster**	**0**	0
Union	(2)	0	0	Loibichl	1	1.66
Vellavia	(2)	0	0	G d velay	3	3.33
Westminster	**(2)**	0	0	Asplund	4	3.33
Dore	(6)	1.66	1.66	Chevalier	6.66	5
G d Velay	(2)	3.33	3.33	Nottingham	7.5	3.33
Armelle	(2)	1.66	1.66	Bigo	13.33	5
Hannchen	(2)	1.66	1.66	Oderbrucker	20	1.66

Table A2: Percentage of leaf area covered with mildew, percentage of infected plants and the percentage of infected leaves under natural infected conditions, glasshouse 2009 (G 2009).

2-7-2009 (first score GS 39-45)				10-7-2009 (second score GS 51-59)				27-7-2009 (third score GS 61-69)					
Average of infected area in one leaf			% plants	Average of infected area in one leaf			% plants	Average of infected area in one leaf			% infected leaves		
Armelle	(2)	0	0	Plumage	(2)	0	0	Plumage	(2)	0	Plumage	(2)	0
Asplund	(6)	0	0	**Tipple**	**(2)**	**0**	0	**Tipple**	**(2)**	**0**	**Tipple**	**(2)**	**0**
Chevalier	(2)	0	0	**Westminster**	**(2)**	**0**	0	**Westminster**	**(2)**	**0**	**Westminster**	**(2)**	**0**
G d Velay	(2)	0	0	Oderbrucker	(6)	1.67	16.66	Union	(2)	15.90	Oderbrucker	(6)	39.78
Nottingham	(2)	0	0	Nottingham	(2)	2.29	61.11	Armelle	(2)	26.28	Vellavia	(2)	50.23
Oderbrucker	(6)	0	0	Vellavia	(2)	2.31	55.55	Oderbrucker	(6)	33.22	Armelle	(2)	56.86
Plumage	(2)	0	0	Union	(2)	2.92	66.66	Vellavia	(2)	34.15	Union	(2)	57.22
Tipple	**(2)**	**0**	0	Asplund	(6)	3.17	61.11	Hannchen	(2)	39.64	Hannchen	(2)	62.21
Vellavia	(2)	0	0	G d Velay	(2)	6.00	16.66	Loibichl	(2)	42.79	Nottingham	(2)	65.16
Westminster	**(2)**	**0**	0	Chevalier	(2)	6.44	72.22	Nottingham	(2)	46.68	Loibichl	(2)	70.21
Loibichl	(2)	0.33	5.55	Hannchen	(2)	8.97	61.11	Chevalier	(2)	51.01	G d Velay	(2)	74.78
Union	(2)	0.33	5.55	Loibichl	(2)	14.17	76.47	G d Velay	(2)	51.38	Asplund	(6)	81.40
Bigo	(6)	1.67	5.55	Dore	(6)	19.25	61.11	Asplund	(6)	52.24	Chevalier	(2)	82.58
Hannchen	(2)	3.00	33.33	Armelle	(2)	20.00	44.44	Dore	(6)	52.51	Bigo	(6)	86.82
Dore	(6)	3.67	16.66	Bigo	(6)	22.77	50	Bigo	(6)	55.77	Dore	(6)	94.78

% infected area second score		% infected area third score		% infected leaves third score	
One-way non-parametric ANOVA		One-way non-parametric ANOVA		One-way non-parametric ANOVA	
P	CV	P	CV	P	CV
< .001	1.22	< .001	.60	< .001	.57

ANOVA analysis of variance, P probability and CV coefficient of variation.

Table A3: Effect of nitrogen levels on mildew disease a-% of infected area, b-% of infected leaves and c-% of infected plants, glasshouse trial, 2010 (G 2010).

a- %of infected	26-5-2010 (first score)					3-6-2010 (second score)					10-6-2010 (third score)				
area.	0.5	1	2.5	5	10	0.5	1	2.5	5	10	0.5	1	2.5	5	10
Armelle (2)	0	0	0	0	0	0	0	0	0	0	0	0	3	5	3.5
Chevalier (2)	4	1	2	1	1	9.58	4.25	2.7	4.38	6.19	12.51	9.32	10.75	14.08	11.66
Oderbrucker (6)	0	0	0	0	0	0	15.5	0	0	0	0	6.42	5.5	4.67	1.8
Plumage (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipple (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Vellavia (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Westminster (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
b-%of infected leaves	0.5	1	2.5	5	10	0.5	1	2.5 a	5 a	10	0.5	1	2.5	5	10
Armelle (2)	0	0	0	0	0	0	0	0	0	0	0	0	14.29	5.67	11.36
Chevalier (2)	28.57	9.09	22.22	9.53	7.69	35.48	10.81	9.63 a	16.05	12.12	28.95	25.29	18.18	17.85	18.78
Oderbrucker (6)	0	0	0	0	0		40				0	33.33	33.33	37.5	33.33
Plumage (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipple (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Vellavia (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Westminster (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
c-%of infected plants	0.5	1	2.5	5	10	0.5	1	2.5	5	10	0.5	1	2.5	5	10
Armelle (2)	0	0	0	0	0	0	0	0	0	0	0	0	12.5	37.5	37.5
Chevalier (2)	14.29	28.57	14.29	28.57	14.29	57.14	28.57	71.43	57.14	85.71	71.43	57.14	100	100	100
Oderbrucker (6)	0	0	0	0	0	0	25	0	0	0	0	37.75	12.5	25	12.5
Plumage (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipple (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Vellavia (2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Westminster (2)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table A4: Percentage of infected plants with FHB under field conditions at Nafferton farm before harvest, 2009 (F 2009).

27-7-2009 (First score)			7-8-2009 (Second score)			19-8-2009 (Third score)		
Armelle	(2)	0	Armelle	(2)	1.67	Armelle	(2)	1.66
Chevalier	(2)	0	Chevalier	(2)	1.66	Chevalier	(2)	1.66
G d velay	(2)	0	**Tipple**	**(2)**	**1.66**	Dore	(6)	5
Nottingham	(2)	1.66	G d Velay	(2)	3.33	G d velay	(2)	5
Tipple	**(2)**	**1.66**	Nottingham	(2)	5	**Tipple**	**(2)**	**6.66**
Union	(2)	1.66	Plumage	(2)	5	Union	(2)	6.66
Westminster	**(2)**	**1.66**	Dore	(6)	5	Bigo	(6)	8.33
Dore	(6)	3.33	Bigo	(6)	6.66	Loibichl	(2)	8.33
Hannchen	(2)	3.33	Oderbrucker	(6)	6.66	Nottingham	(2)	8.33
Loibichl	(2)	3.33	Union	(2)	6.66	Vellavia	(2)	10
Bigo	(6)	5	**Westminster**	**(2)**	**8.33**	Asplund	(6)	11.66
Oderbrucker	(6)	5	Loibichl	(2)	8.33	Hannchen	(2)	11.66
Plumage	(2)	5	Vellavia	(2)	10	Oderbrucker	(6)	11.66
Asplund	(6)	6.66	Asplund	(6)	10	Plumage	(2)	11.66
Vellavia	(2)	8.33	Hannchen	(2)	10	**Westminster**	**(2)**	**11.66**

27-7 One-way non-parametric ANOVA		7-8 One-way parametric ANOVA				19-8 One-way non-parametric ANOVA	
P	CV	*P*	df	F	CV	*P*	CV
. 072	1.10	.376	14, 30	1.127	0.83	.195	.684

ANOVA analysis of variance, P probability, CV coefficient of variation, df degree of freedom and LSD least significant difference.

Appendix.

Table A5: Percentage of infected grains and heads with *F. culmorum* under glasshouse conditions in Sunderland before harvest, 2010 (G 2010).

% of infection	16/7/2010 (first score)						
	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
% grain.	1.14	0	1.36	0	**1.41**	4.42	**1.73**
% heads	9.56	0	17.39	0	**10.91**	25	**14.41**
	23/7/2010 (second score)						
% of infection	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
% grain.	6.67	0.93	7.78	0.59	**10.35**	13.59	**6.71**
% heads	41.18	7.69	38.64	18.52	**42.74**	48.09	**27.42**
	30/7/2010 (third score)						
% of infection	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
% grain.	13.58	4.02	7.63	1	**19.83**	20.19	**9.38**
% heads	54.84	18.31	31.82	7.89	**51.22**	46.67	**40**

% grain infection 16-7-2010					% head infection 16-7-2010				
One-way non-parametric ANOVA					One-way non-parametric ANOVA				
P	CV				P				CV
< .001	1.21				< .001				0.91
% grain infection 23-7-2010					% head infection 23-7-2010				
One-way parametric ANOVA					One-way non-parametric ANOVA				
P	df	F	CV	LSD	P				CV
< .001	6, 63	127.225	0.68	1.175	< .001				0.60
% grain infection 30-7-2010					% head infection 30-7-2010				
One-way non-parametric ANOVA					One-way non-parametric ANOVA				
P	CV				P	df	F	CV	LSD
< .001	0.66				< .001	3, 63	37.223	0.52	8.096

ANOVA analysis of variance, P probability, CV coefficient of variation, df degree of freedom and LSD least significant difference

Table A6: Incidence of *F. culmorum* infection (Percentage of infected heads) in barley grown under different levels of nitrogen before harvest: a- first score, b- second score and c- third score (N3).

N mM	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
a- 16/7/2010 (first score)							
0.5	0	0	25	0	**0**	0	**0**
1	0	0	0	0	**0**	0	**5.55**
2.5	0	0	0	0	**0**	0	**0**
5	0	0	16.67	0	**0**	0	**0**
10	0	0	0	0	**0**	0	**0**
b- 23/7/2010 (second score)							
	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
0.5	0	0	25	0	**30.77**	0	**18.75**
1	0	0	0	0	**39.13**	12.5	**22.22**
2.5	11.11	0	33.34	0	**10.71**	40	**8**
5	33.33	10	37.5	16.67	**7.69**	30	**15.73**
10	61.54	0	57.14	0	**28**	58.33	**12.9**
c- 30/7/2010 (third score)							
	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
0.5	0	0	25	0	**38.46**	0	**37.5**
1	0	0	0	25	**66.67**	14.29	**44.44**
2.5	33.33	0	33.33	33.33	**56.25**	41.67	**25.93**
5	50	10	49.91	30	**24**	75	**28.57**
10	41.67	10	44.44	0	**41.62**	42.11	**46.66**

	23-7-2010 (second score) One-way non-parametric ANOVA		30-7-2010 (third score) One-way non-parametric ANOVA	
	P	CV	*P*	CV
V	< .001	1.38	< .001	.98
N	.016		.015	

ANOVA analysis of variance, P probability, CV coefficient of variation, V between varieties and N between N levels

Appendix.

Table A7: Incidence of *F. culmorum* infection (Percentage of infected grains) in barley grown under different levels of nitrogen before harvest. a- first score, b- second score, and c- third score (N3).

N mM	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
	Barley varieties/ Row type						
			a- 16/7/2010 (first score)				
0.5	0	0	7.33	0	**0**	0	**0**
1	0	0	0	0	**0**	0	**1.71**
2.5	0	0	0	0	**0**	0	**0**
5	0	0	1.61	0	**0**	0	**0**
10	0	0	0	0	**0**	0	**0**

b- 23/7/2010 (second score)

	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
0.5	0	0	8.9	0	**30.30**	0	**15.91**
1	0	2.27	0	0	**10.04**	2.08	**6.15**
2.5	4.69	0	3.75	0	**7.11**	20.47	**6.94**
5	9.97	0	32.60	2.27	**13.96**	31.67	**25.48**
10	41.68	0	45.40	0	**19.97**	48.31	**11.13**

c- 30/7/2010 (third score)

	Armelle (2)	Chevalier (2)	Oderbrucker (6)	Plumage (2)	Tipple (2)	Vellavia (2)	Westminster (2)
0.5	0	0	9.57	0	**29.92**	0	**18.18**
1	0	0.46	0	1.32	**18.19**	2.08	**20.37**
2.5	9.58	0	2.5	10.87	**14.20**	23.30	**17.18**
5	29.68	0	32.92	5.68	**29.17**	56.99	**31.77**
10	45.70	0.23	48.31	0	**60.51**	85.42	**50.82**

	23-7-2010 (second score) One-way non-parametric ANOVA		30-7-2010 (third score) One-way non-parametric ANOVA	
	P	CV	*P*	CV
V	< .001	1.70	< .001	1.30
N	< .001		< .001	

ANOVA analysis of variance, P probability, CV coefficient of variation, V between vVarieties and N between N levels

Table A8: Effect of nitrogen levels on tiller number in modern and historic barley varieties before harvest.

Variety /Row type		Nitrogen levels (mM)				
		0.5	1	2.5	5	10
Armelle	(2)	1.13	2	3	4	5.38
Chevalier	(2)	2.5	3	5	6.75	10.25
Oderbrucker	(6)	1.63	1.38	2	2.25	4.25
Plumage	(2)	1	1	1.75	3.25	5
Tipple	**(2)**	**4**	**5**	**6.29**	**7.42**	**12.43**
Vellavia	(2)	2	2.5	4.25	7.25	9.5
Westminster	**(2)**	**3.71**	**4.29**	**6**	**8.42**	**11.29**

One-way non-parametric ANOVA		
	P	CV
V	< .001	.72
N	< .001	

ANOVA analysis of variance, P probability, CV coefficient of variation, V between Varieties and N between N levels.